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EDITED BY

SIMON FLEXNER, M.D.

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THE EFFECT OF CARBON DIOXIDE IN THE CULTIVATION OF THE MENINGOCOCCUS.

By FREDERICK L. GATES, M.D.

(*From the Laboratories of The Rockefeller Institute for Medical Research.*)

PLATES 15 AND 16.

(Received for publication, January 29, 1919.)

The experiments here reported are the outcome of observations made in applying the recommendation of Cohen and his associates[1,2,3] that meningococci be grown at a partial oxygen tension, obtained by substituting carbon dioxide for approximately 10 per cent of the air in a closed container.

The moisture requirement of the meningococcus has been repeatedly emphasized,[4] and it seemed important to determine whether Cohen's results might not be due, in part at least, to the retention of moisture in the partial tension chambers. In our series of carrier examinations two sets of carrier plates were incubated in moist chambers; one set in air, the other in the presence of carbon dioxide, from 2 to 25 per cent by volume. Our uniform experience has been that nasopharyngeal strains of meningococci grow as well, or better, in air saturated with water vapor in a closed chamber as in a similarly saturated atmosphere containing a small percentage of carbon dioxide. The conditions governing the luxuriance of the growth are factors of the humidity of the chamber and the qualities of the medium, and not of the slight differences in oxygen tension resulting from the replacement of a small part of the air with carbon dioxide.

Recently the opportunity presented itself at Camp Zachary Taylor of making similar comparisons with growths of meningococci

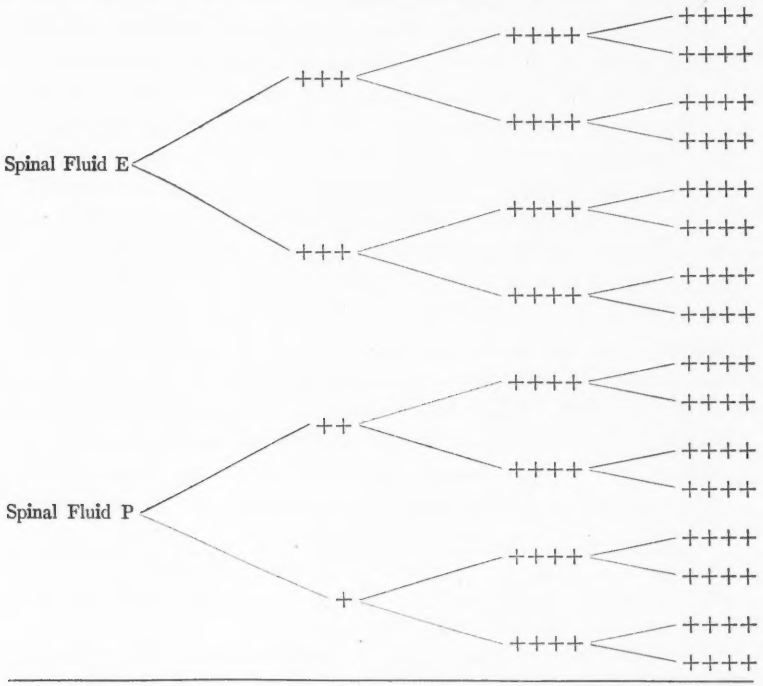
[1] Cohen, M. B., and Markle, L., A method which greatly facilitates the culture of the meningococcus, *J. Am. Med. Assn.*, 1916, lxvii, 1302.

[2] Cohen, M. B., Cultivation of the meningococcus under partial oxygen tension, *J. Am. Med. Assn.*, 1918, lxx, 1999.

[3] Cohen, M. B., and Fleming, J. S., The diagnosis of epidemic meningitis and the control of its treatment by rapid bacteriologic and serologic methods, *J. Infect. Dis.*, 1918, xxiii, 337.

[4] Lloyd, D. J., On vitamines, amino-acids, and other chemical factors involved in the growth of the meningococcus, *J. Path. and Bacteriol.*, 1916–17, xxi, 113.

from spinal fluids. As a routine approximately 2 cc. of spinal fluid were added to each of six slants of medium.[5] Three tubes of each set were incubated in a water-sealed chamber, in air, while three were incubated in a similar chamber containing 10 per cent carbon dioxide. No significant difference could be observed in the growths under these conditions. The slight differences noted favored the chamber containing air alone. Successive generations were also grown alternately in air and in 10 per cent carbon dioxide, without visible diminution in luxuriance. Two typical strains may be charted by the scheme by which Cohen has illustrated his results. Upstrokes indicate cultures in air, downstrokes cultures in 10 per cent carbon dioxide. One plus sign indicates a single colony, two indicate several colonies, three many, and four a confluent growth.



[5] Veal infusion agar, with glucose 1 per cent, adjusted to pH 7.4, to which 5 per cent sterile, unheated, citrated horse plasma was added just before tubing. This is a medium highly favorable to the meningococcus.

From these and similar findings the conclusion was reached that the replacement of 10 per cent of air by carbon dioxide has no appreciable effect on the growth of either nasopharyngeal or spinal strains of meningococci, under the conditions of our experiments.

Cohen considers that the effect of carbon dioxide, as he describes it, is not specific for this gas: that it acts merely as a neutral agent, displacing oxygen, and he calls the meningococcus a "microaerophile." A brief consideration of the composition of air, and of the actual displacement of oxygen by 10 per cent carbon dioxide shows that only approximately 2 per cent of oxygen would be displaced and its concentration reduced from 20 per cent to 18 per cent. Such a slight diminution could hardly be expected to produce the marked effects that Cohen reports. Moreover, if the action of carbon dioxide were not specific, results similar to Cohen's should be obtained with any neutral gas, such as nitrogen, which is chemically so inert. The fact that air is composed largely of nitrogen, and that only peculiar species of bacteria are able to utilize nitrogen from the air would seem to make it the most desirable agent for replacing oxygen in testing various oxygen tensions. Therefore nitrogen was used to replace oxygen in the following experiment.

Experiment 1.—January 13, 1919.

Eight plates of veal infusion agar, pH 7.4, with 5 per cent fresh sterile rabbit serum were inoculated with a suspension of a recently isolated spinal strain of normal meningococcus. Two of these plates were placed in each of four large Novy jars containing a little water. In three of the jars concentrations of nitrogen of 10, 25, and 50 per cent, in addition to that already present in the air, were obtained by partial exhaustion of the air and replacement with the gas from a tank. Thus partial oxygen tensions of approximately 18, 15, and 10 per cent were produced in the jars. The fourth jar served as an air control.

After 16 hours incubation the growths of meningococcus were profuse and practically identical in air, in 18 per cent oxygen, and in 15 per cent oxygen. Luxuriant confluent streaks of organisms showed the path of the inoculation. On one of the plates in 10 per cent oxygen the growth was poor, in the other very poor. Only a few small confluent masses of growth showed the site of heaviest inoculation. A plate from each jar is reproduced in Plate 15, Petri Plates 5 to 8.

The meningococcus did not thrive on artificial medium when deprived of half its accustomed oxygen tension. But this experiment does

not indicate that variations of 2 to 5 per cent below normal make any essential difference in its growth. What effect would increased oxygen tension have?

Experiment 2.—January 14, 1919.

Plates similar to those used in Experiment 1 and inoculated as before were divided among the four Novy jars. In a similar manner oxygen was used to replace the exhausted air in percentages of 5, 15, and 25, making the oxygen tensions of the atmospheres in the jars approximately 24, 32, and 40 per cent. The fourth jar again contained air alone.

After 16 hours incubation the growths in all the jars were profuse. Beside the confluent growths where the plates were most heavily inoculated large single colonies of luxuriantly growing organisms appeared. The meningococcus grew as well in 40 per cent oxygen as in air (Plate 15, Petri Plates 1 to 4).

Provided the oxygen tension is sufficient to support its growth, the meningococcus does not seem to be exacting about the concentration of its oxygen supply. These experiments show that the effects of increased carbon dioxide tension, as described by Cohen, are not due to the displacement of oxygen and that the meningococcus is not a "microaerophile." The action of carbon dioxide must be inherent in some specific property of the gas. Carbon dioxide in solution acts as a weak acid and might have a chemical effect on the medium in which meningococci are grown and on the organisms themselves. If serum agar plates, such as are used for isolating the meningococcus, are incubated in a moist atmosphere containing 10 to 30 per cent carbon dioxide, their reaction may be shifted toward the acid end of the pH scale. The following experiment shows this reaction and its effect upon a recently isolated (fourth generation) spinal strain of a normal meningococcus.

Experiment 3.—January 3, 1919.

Four 100 cc. lots of veal infusion agar were adjusted to the following reactions.

	Titratable acidity (phenolphthalein). *per cent*	pH concentration (phenolsulfonephthalein).
Lot 1	1.6	7.2
" 2	0.8	7.8
" 3	0.4	7.9
" 4	0.3	8.0+

To each lot were added 5 cc. of sterile, unheated rabbit serum, and eight plates were poured. Four plates of each set were heavily inoculated with a

suspension of meningococci from a third generation slant of a spinal strain. Eight plates (one inoculated and one sterile from each lot) were placed in each of four large Novy jars. In three of the jars concentrations of carbon dioxide of 10, 20, and 30 per cent were obtained by partial exhaustion of the air and replacement with the gas from a tank. The fourth jar served as an air control. After 18 hours incubation at 37.5°C. the growths of meningococci were noted and the pH concentrations of the uninoculated plates read with phenolsulfonephthalein and cresol purple as the indicators.

TABLE I.

Set No.	Original titer.		Final titer (pH).				Growths.			
	Acidity.	pH	Air.	Carbon dioxide.			Air.	Carbon dioxide.		
				10 per cent.	20 per cent.	30 per cent.		10 per cent.	20 per cent.	30 per cent.
	per cent									
1	1.6	7.2	7.4	7.0	6.7	6.4	Profuse.	Profuse.	Profuse.	Fair.
2	0.8	7.8	8.0	7.4	6.8	6.6	Good.	"	"	"
3	0.4	7.9	8+	7.4	6.8	6.6	None.	"	"	Good.
4	0.3	8+	8+	7.6	6.8	6.7	"	"	"	Profuse.

Table I shows the results, and Plate 16 the growths obtained in air, in 10 per cent carbon dioxide, and in 30 per cent carbon dioxide. The growths in 10 and 20 per cent carbon dioxide were practically identical. Several points are to be noted in an analysis of these findings.

The meningococcus does not grow in a medium of a titratable acidity of +0.4 or less when this corresponds to a pH value greater than 7.8 to 8.0 on the Sörensen scale. It may grow luxuriantly on a medium with a titratable acidity of +1.6 when this corresponds to pH 7.2. The fallibility of the old method of titrating media is clearly seen.

The partial saturation of a medium with carbon dioxide from an atmosphere containing 10 to 30 per cent of the gas may increase its hydrogen ion concentration markedly. Almost regardless of the original hydrogen ion concentration, the final reaction seems to represent a state of equilibrium between the carbon dioxide in the medium and in the surrounding air. Thus in the present experiment 10 per cent carbon dioxide brought the more alkaline plates to ap-

proximately pH 7.4 to 7.6, 20 per cent brought them to pH 6.8, and 30 per cent brought them to pH 6.6 to 6.7.

It is evident that such an action of carbon dioxide on the medium might exert either a favorable or an unfavorable effect upon meningococci, according to the original reaction of the medium. A favorable effect is seen in Petri Plates 3 and 4 of all the carbon dioxide series—a medium too alkaline to support growth was brought to a hydrogen ion concentration favorable to the growth of the meningococcus. An unfavorable effect, on the other hand, is seen in Petri Plates 1 and 2 of the carbon dioxide 30 per cent series; the medium was rendered too acid, and the meningococcus grew less well than in air.

Cohen's contention that meningococci which have developed aerobically do not grow well in the presence of carbon dioxide and *vice versa* is not borne out by this experiment, in which a strain isolated and grown for three generations aerobically grows well in carbon dioxide on a medium in which it cannot grow in air. In connection with the first experiment it may be pointed out that a meningococcus isolated and subcultured in air grew equally well under slightly reduced oxygen tension.

Cohen's reports indicate that this effect of carbon dioxide on the medium has operated favorably in his experiments, and thus the discrepancy between his results and ours may be explained. He and Fleming[3] describe the use of media 0.2 to 0.3 per cent acid to phenolphthalein, under the impression "that the meningococcus will not usually grow when the reaction is over plus 0.5 [per cent] acid to phenolphthalein." In serum agar, 0.2 to 0.3 per cent acid usually corresponds to pH 8.2 to 7.8 on the Sörensen scale, according to the amount of buffer present. Kligler[6] has recently found that the range of growth of the meningococcus in serum dextrose broth is pH 6.1 to 7.8, with the optimum at pH 7.4, and this fact, in conjunction with the effect of carbon dioxide in shifting the reaction toward the acid side, as shown above, suggests that incubation in a partial tension of carbon dioxide has made Cohen's media less unfavorable to the meningococcus, and so, combined with the undoubted aid of

[6] Kligler, I. J., personal communication.

moisture in the closed container, produced the results that he describes.

As for the effect of carbon dioxide on the organisms themselves, a specific action is not so easily demonstrated. That partial tensions up to 30 per cent may not be injurious is shown by the growths on the less acid plates of this series. On the other hand, Shaw-MacKenzie[7] states that carbon dioxide, 22 volumes per cent in Ringer-Locke solution, causes the death of the meningococcus in 20 minutes and suggests that "even the CO_2 normally occurring in the plasma and body fluids may form part of the protective processes of the body."

SUMMARY.

The meningococcus is not a "microaerophile." It grows equally well in atmospheres containing from 15 to 40 per cent oxygen.

If small amounts of carbon dioxide affect the growth of the meningococcus on an artificial medium it is by changing the reaction of the medium, not by slightly reducing the oxygen tension of the surrounding air.

The fallibility of titrating the total acidity of a medium is again clearly demonstrated. A reaction favorable to the meningococcus cannot be determined from the total titratable acidity but depends solely upon the hydrogen ion concentration of the medium. The optimum for the meningococcus is approximately at pH 7.4.

The value of a moist chamber in the cultivation of the meningococcus is shown by unusually luxuriant growth when other conditions are also favorable.

Addendum.

Since the completion of these experiments and the receipt of this paper for publication, St. John (St. John, J. H., *Med. Rec.*, 1919, xcv, 184) has reported his experiments on "Oxygen tension in its relation to the meningococcus." He concludes that the oxygen tension factor is at least of minor importance in comparison with the effect of moisture in promoting the growth of meningococci. He calls

[7] Shaw-MacKenzie, J. A., Toxic action of carbonic and other weak acids on the meningococcus, *J. Roy. Army Med. Corps*, 1918, xxxi, 1.

attention to the fact that the meningococcus is known to be especially sensitive to the degree of moisture and the reaction of the medium, and suggests that "the reaction of the meningococcus medium may be favorably influenced by certain gases evolved by the growing *B. subtilis*, but this consideration is also minimized by comparison with moisture controls."

At the time that St. John's article appeared there was in press a paper by Frederick L. Gates and Edgar T. H. Tsen on "The effect of moisture on the growth of the meningococcus" which was to have appeared in this number of *The Journal of Experimental Medicine*. Our findings on the importance of moisture in the cultivation of the meningococcus merely corroborated St. John's, and added nothing but emphasis to his conclusions. Our paper was therefore withdrawn from publication. We feel, however, that the degree of humidity most favorable to the meningococcus is not to be obtained easily unless closed containers are employed.

EXPLANATION OF PLATES.

PLATE 15.

The effect of different oxygen tensions on the growth of the meningococcus.

Petri Plates 1 to 3. 16 hour growths of a spinal strain under increased oxygen tension. Petri Plate 4. A control growth in air.

Petri Plate 5. A control growth in air. Petri Plates 6 to 8. 16 hour growths of a spinal strain under decreased oxygen tension.

PLATE 16.

The effect of carbon dioxide on the growth of the meningococcus, caused by changing the hydrogen ion concentration of the medium.

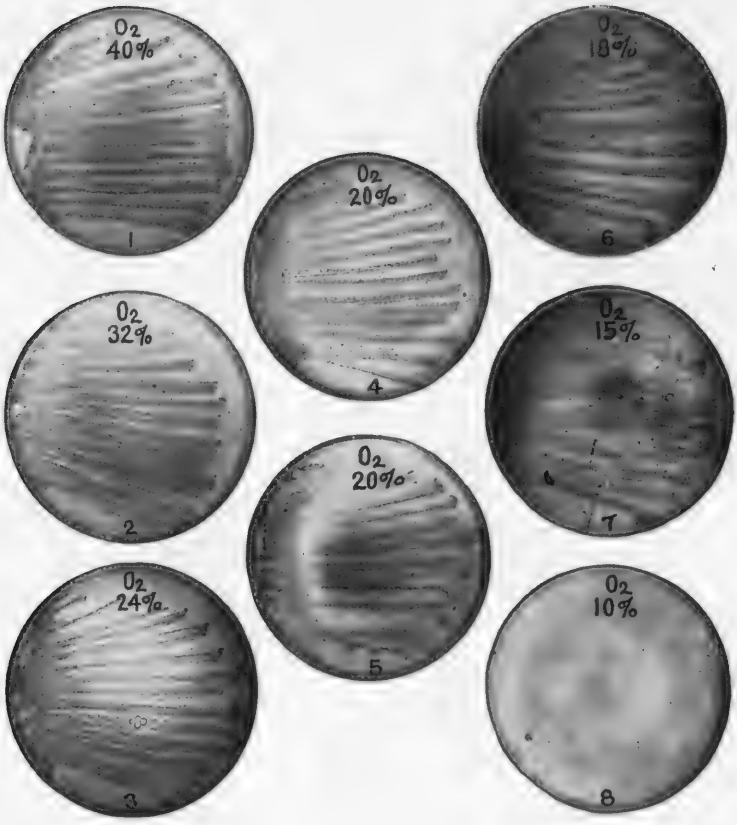
Petri Plates 1, 1, 1. Original pH 7.2; titratable acidity 1.6 per cent.

Petri Plates 2, 2, 2. Original pH 7.8; titratable acidity 0.8 per cent.

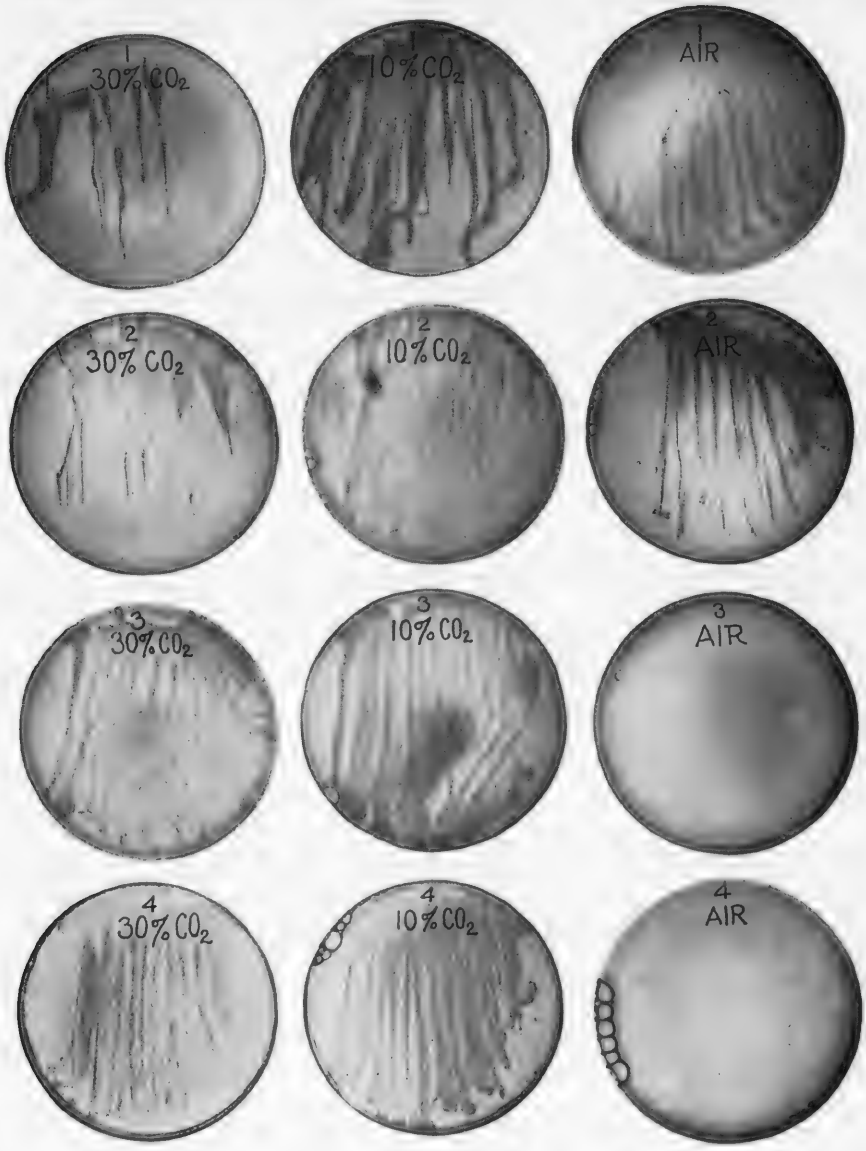
Petri Plates 3, 3, 3. Original pH 7.9; titratable acidity 0.4 per cent.

Petri Plates 4, 4, 4. Original pH 8+; titratable acidity 0.3 per cent.

In Sets 1 and 2, 30 per cent carbon dioxide increased the hydrogen ion concentration beyond the zone favorable to growth. In Sets 3 and 4, 10 and 30 per cent carbon dioxide brought plates originally too alkaline to support growth into the zone of hydrogen ion concentration favorable to the growth of the meningococcus.



(Gates: Cultivation of meningococcus.)



(Gates: Cultivation of meningococcus.)

CICATRIZATION OF WOUNDS.

X. A General Equation for the Law of Cicatrization of Surface Wounds.

By P. LECOMTE du NOÜY, D.Sc.

(From the Laboratories of The Rockefeller Institute for Medical Research, New York, and Hospital 21, Compiègne, France.)

(Received for publication, August 8, 1918.)

It has been shown[1] by the extrapolation formula

$$S_n = S_{n-1}\left[1 - i\left(t + \sqrt{t + nt}\right)\right]$$

that the normal progress of cicatrization of surface wounds follows a definite curve. The fact that many biological and chemical phenomena are expressed by exponential formulas suggested the comparison, if possible, of the curve for the cicatrization of wounds with other curves expressing biological phenomena. It is well known that the exponential function plays an important part in natural phenomena. It expresses the general law called by Lord Kelvin "the compound interest law," and by Mellor, the "ubiquitous law."

I had already studied a formula of the form

$$y = \frac{K}{x - a} \quad \text{(hyperbola)}$$

which was suggested to me by Professor Houssay, by means of which he expresses the phenomenon of regression of certain organs in animals, under special conditions; but this proved to be unsuccessful.

On the basis that during the short time dt the cicatrized area ds remains proportional to the total area, we can write

$$-ds = KSdt \tag{1}$$

[1] du Noüy, P. L., *J. Exp. Med.*, 1916, xxiv, 451, 461; 1917, xxv, 721.

by integration in respect to time,

$$T = -\int_{S_o}^{S} \frac{ds}{KS} \tag{2}$$

or

$$T = -\frac{1}{K}\int_{S_o}^{S} \frac{ds}{S}$$

hence

$$T = \frac{1}{K}\ \mathrm{Log}_e \frac{S_o}{S}$$

which is similar to the equation of Slater,[2]

$$T = \frac{1}{K}\ \mathrm{Log}_e \frac{N+n}{N}$$

and finally,

$$KT = \mathrm{Log}_e \frac{S_o}{S} \tag{3}$$

that is,

$$S = S_o e^{-KT}$$

We can then compute the values of the coefficient K for the different values of T. K increases regularly. Therefore, the curve obtained from the equation

$$S = S_o e^{-KT}$$

does not correspond to the facts, and gives for every value of T a certain value of S which deviates more and more from that calculated according to formula (1) (extrapolation form). We were then obliged to introduce a new coefficient, stating the problem in the following way: Is it better to attempt to find this new coefficient by giving to T its real value and by studying the variations of K, or is it more advisable to study the variations of the exponent if K remains

[2] Slater, A., *Biochem. J.*, 1912–13, vii, 197.

constant; that is, the variations of a certain coefficient α as in the exponent

$$-K(T+\alpha) \tag{4}$$

The study of a large number of cases showed that by trying to find the correction of the coefficient K, I encountered a practical difficulty from the fact that since this coefficient is small in respect to T, the smallest numerical variations such as those arising from calculation errors with 2 or 3 decimal numbers were of sufficient importance to destroy the concordance of the curves. On the contrary, in the second case, fairly important variations in a certain coefficient K_2, the connection of which with α can be expressed as

$$\alpha = \frac{T^2}{K_2}, \tag{5}$$

interfered very little with the accuracy of the calculation.

Text-fig. 1 shows the variations of the coefficient K in function of time. The angular coefficient of the lines seems to vary proportionally with the index of cicatrization, as

$$\text{index } i = \frac{S_o - S}{S_o\,(t+1\sqrt{t})}$$

It is by no means certain that these lines are straight lines mathematically (see the straight dotted line in Text-fig. 1), but the observations are limited by time and it is difficult to determine this point. In this chart the value of K is given by equation (3) from which the following formula is obtained:

$$K = \frac{\text{Log } S_o - \text{Log } S}{T} \tag{6}$$

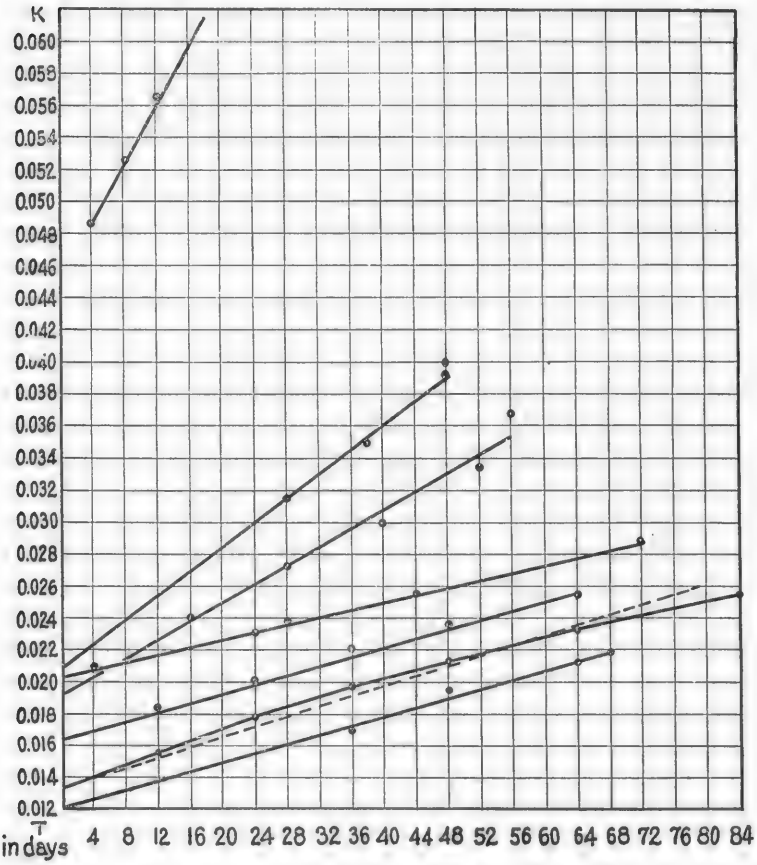
Text-fig. 2, on the contrary, shows the variations of the coefficient α previously determined, the value of which is

$$\alpha = \frac{\text{Log } S_o - \text{Log } S}{K} - T \tag{7}$$

By plotting in ordinates the values of α which represent the difference between the curve resulting from equation (3) and that resulting

from equation (1), we obtain a curve which expresses the law of these differences. It is a branch of parabola and the equation is

$$y^2 = 2\,px$$



TEXT-FIG. 1. Variations of the coefficient K, in function of time.

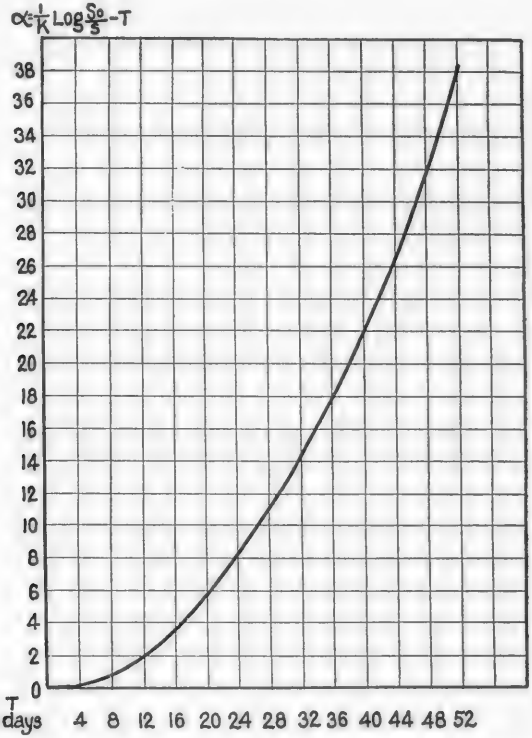
That is, by replacing the letters by those we have adopted, *viz.*, $y = T$, and $x = \alpha$,

(8) $$\alpha = \frac{T^2}{2p}$$

The significance of the coefficient K_2 in equation (5) appears now clearly, and the equation may be written

$$S_T = S_o e^{-K\left(T+\frac{T^2}{2p}\right)} \tag{9}$$

which is the general equation of the law.



TEXT-FIG. 2. Variations of the coefficient α in function of time in the formula $S_T = S_o e^{-K(T+\alpha)}$

Before we begin a thorough study of the coefficients it may be interesting to compare, for example, two series of figures representing the ordinates, *viz.* the areas of wounds in square centimeters, of two

cicatrization curves obtained, the first (figures of the upper row) by means of the last exponential equation (9), the second (figures of the lower row) by means of the former extrapolation formula (1). It is obvious that the concordance is almost perfect and that the differences are beyond the errors of experimentation (Table I).

These two examples suffice to show that the proposed equation fulfills the required conditions; in all the cases the coincidence is equally satisfactory. Slight differences, however, sometimes may be observed at the beginning of the curve (for $T = 4$, 8, 12 days), but since the exponential equation has been mathematically studied in a different manner from the first formula, and since, on the other hand, these differences may be affected by errors of measure of the area of wounds, it cannot be concluded that the equation previously proposed is more accurate than the new one.

Study of the Coefficients K and 2p.

As the coefficient K can be determined within 4 days, that is from two points on the curve, 4 days apart, and as the contraction, especially for the large wounds, plays the principal part at the beginning of cicatrization, this coefficient characterizes the contraction, and during the first days the relative rate of repair, with reference to the total area of the wound. But it has been stated[1] that this rate is itself a function of the age of the man, within certain limits. Hence the coefficient K must logically be proportional to the index of cicatrization i which plays the same part in formula (1). The calculation of a number of curves shows that this is so.

The velocity of repair is originally determined by the area of the wound. We have assumed that at the beginning of the phenomenon it remained proportional to the area for a very short time. We proceeded from this assumption to state the differential equation

$$-ds = KSdt$$

If the velocity remained proportional to the area, this would explain the increasing delay due to the reduction of the area of the wound. On account of this delay, the phenomenon is expressed by a logarithmic curve and not by a straight line, for, at a certain moment T, the area which is not yet cicatrized is $TM = S$ (Text-fig. 3).

TABLE I.

Area calculated with.	Time in days.																			
	0	4	8	12	16	20	24	28	32	36	40	44	48	52	56	60	64	68	72	76
Patient 263.																				
	sq. cm.	*sq. cm.*	*sq. cm.*	*sq. cm.*	*sq. cm.*	*sq. cm.*	*sq. cm.*	*sq. cm.*	*sq. cm.*	*sq. cm.*	*sq. cm.*	*sq. cm.*	*sq. cm.*	*sq. cm.*	*sq. cm.*	*sq. cm.*	*sq. cm.*	*sq. cm.*	*sq. cm.*	*sq. cm.*
Equation (9)	61.8	50.8	41.4	33.4	26.8	21.2	16.7	13.1	10.2	7.7	6.0	4.5	3.4	2.5	1.8	1.4	1.0	0.7	0.5	0.4
" (1)	61.8	51.0	41.6	33.6	26.9	21.3	16.7	13.1	10.2	7.7	6.0	4.5	3.4	2.5	1.8	1.4	1.0	0.7	0.5	0.4
Patient 706.																				
Equation (9)	27.4	21.8	17.1	13.4	9.9	7.2	5.1	3.6	2.4	1.6	1.1	0.69	0.43							
" (1)	27.4	22.0	17.1	13.0	9.6	7.2	5.1	3.6	2.4	1.6	1.1	0.7	0.46							

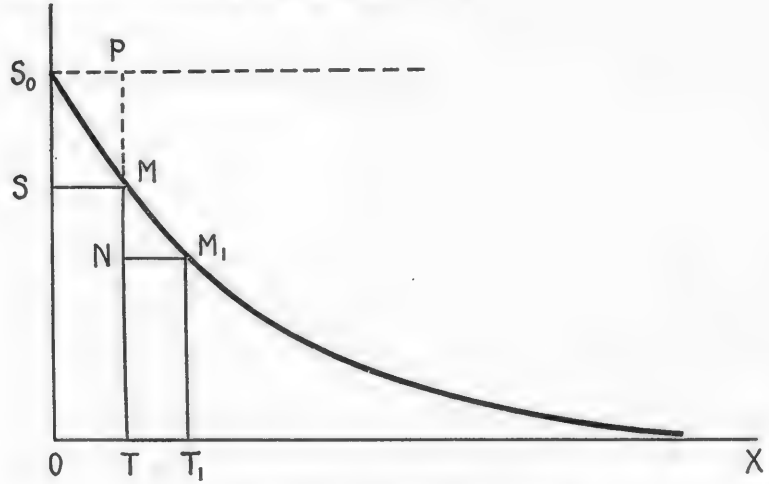
The surface which is already cicatrized is represented by

$$MP = S_0 - S$$

The law of the curve, if logarithmic, is that the decrease MN of the ordinate S, when passing from the time T to the time T_1, is proportional to the length S of the ordinate; that is, the area cicatrized during the time $T_1 - T$ is proportional to the area which is not yet cicatrized. This is what we have written in mathematical symbols, for infinitesimal values

$$-ds = KSdt$$

for ds corresponds to MN and dt to $T_1 - T$.



TEXT-FIG. 3. Logarithmic curve.

But this hypothesis, true at the beginning of the phenomenon, under certain conditions, grows rapidly erroneous, since we have stated that the curve resulting from this equation deviates more and more from the experimental facts. Hence the diminution of the area is not the only factor which governs the real curve. A careful study of the latter and a comparison with the plain logarithmic curve shows that to the *decreasing* acceleration a uniformly *increasing* acceleration is opposed, which at every moment counteracts the effect of the delay due to the decrease of the area.

But if the hypothesis is justified at a certain moment, the simple equation which proceeds from it

$$S_T = S_0 e^{-KT}$$

must represent the phenomenon at the beginning and must express the part played by the first factor, the contraction, which intervenes alone at this moment, as long as the second disturbing factor does not enter into action, or its part is small with reference to that of the first one.

We can verify the correctness of this statement by drawing the curve representing the contraction of a wound; this can be done by measuring the total area of the new scar tissue, no longer merely the area of granulations. For we know that the decrease of this area measures solely the contraction.[3] Then the contraction curve obtained in this way should logically, within certain limits, comply with the law expressed by equation (3). Text-fig. 4 illustrates this fact, and our first hypothesis was therefore justifiable.

It is easily seen that the phenomenon follows the law until, owing to the decrease of the wound area, a more important part of the work of reparation in respect to the area of the granular surface is carried out by the second factor. Then, the decrease of the area being much greater than indicated by equation (3), the contraction, which depends obviously on the area not yet cicatrized, slackens gradually, until it ceases entirely. These observations would show plainly, if we were not already aware of it, that the second factor is the epithelization, and it is then understood that its action is represented in equation (9) by the quotient $\frac{T^2}{2p}$, which expresses that its efficiency, feeble at the beginning, increases slowly at first, then more rapidly, according to a parabolic law.

The above statements are generally verified only if the first observations are made when the cicatrization has already begun, and little or no epithelization has yet appeared. The starting-points of both curves (contraction and cicatrization) are confounded, that is they have the same ordinate at the time 0, so that the surface of the wound itself and that of the cicatrix cannot be discerned from each

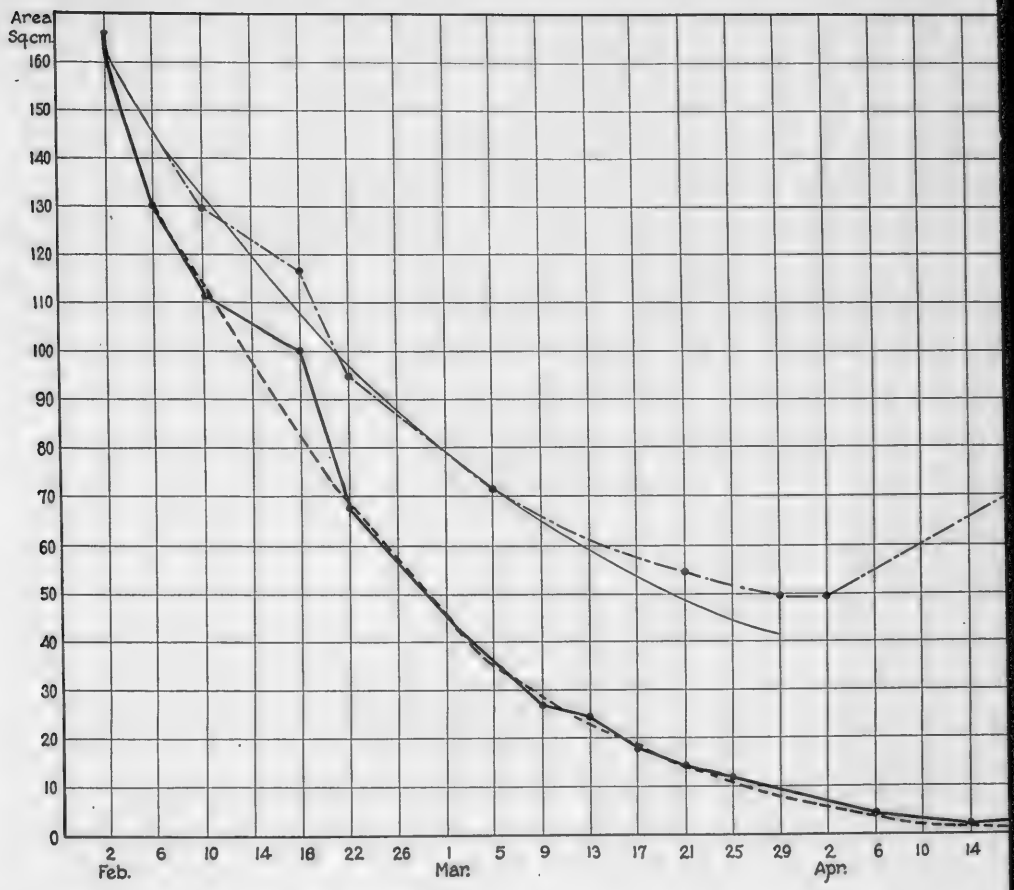
[3] Carrel, A., and Hartmann, A., *J. Exp. Med.*, 1916, xxiv, 429.

other, the edges of the wound being constituted by the old skin or a new and hardly visible epithelial border (Text-fig. 4). When epidermization has already begun, the ordinates at the time 0 are not coincident. If S_o is the ordinate of the area of granulations, in square centimeters, and S_1 the area of the cicatrix, let us call A the difference $S_o - S_1$. A represents the surface already covered by the new epithelium and the equation becomes

$$S_1 = A + S_o e^{-KT}$$

$A + S_o$ is what we have called the area of the cicatrix. But in this case it is often more difficult to verify formula (3) for the contraction, because the epithelization may have become important enough to disturb the simple phenomenon of contraction, the disturbing action being obviously the function of A. The difficult definition of the outline of the cicatrix is also a cause of error. This explains why it is difficult, except on experimental wounds, to find cases on which observation can be made accurately. However, Text-fig. 5 shows that this is possible. The measure of the cicatrix area is made by drawing on cellophane the common limit of the old skin and of the new epithelium, or scar tissue. It is essential to draw this outline on the skin itself, in order to prevent errors of interpretation and of drawing which are frequent, as this common limit often lacks sharpness. But if at the beginning it is tattooed (on animals) or drawn with a dermographic pencil (on men), the measures become comparable and can be done with sufficient accuracy. Every time a drawing is taken, it is advisable to go over the outline again with the pencil where it shows a tendency to be obliterated.

As regards the term $\frac{T^2}{2p}$, what has already been said concerning its growing action in function of time must be taken merely from a mathematical standpoint and not as an assumption dealing with the mechanism of the phenomenon itself. The activities of the real factors are not known, and we can only measure one of the results of these activities, which may vary proportionally to the mathematical factors. Our knowledge does not go beyond that. For example, we know that $\frac{T^2}{2p}$ increases slowly at first, then rapidly, and we assume



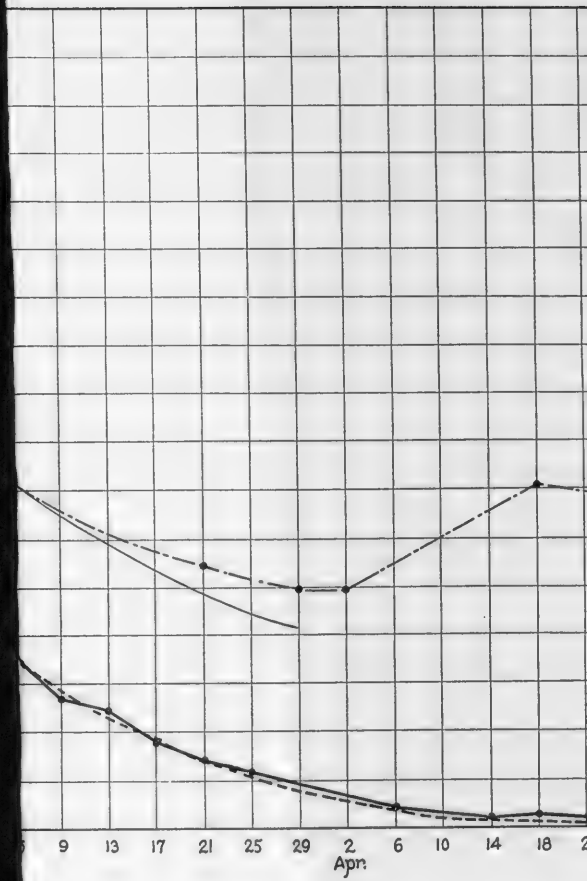
TEXT-FIG. 4. Patient 360. The dotted and broken line represents the contraction of the wound (for Carrel and Hartmann[3]). The light line is the calculated curve, according to the formula

$$S_T = S_o e^{-KT}$$

The heavy line represents the decrease of the area of the wound (curve of cicatrization), and the dotted li according to the equation

$$S_T = S_o e^{-K\left(T + \frac{T^2}{2p}\right)}$$

The decrease in the rate on February 18 is due to infection.



en line represents the contraction of the wound (for detail
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$$S_T = S_o e - KT$$

the wound (curve of cicatrization), and the dotted line, th

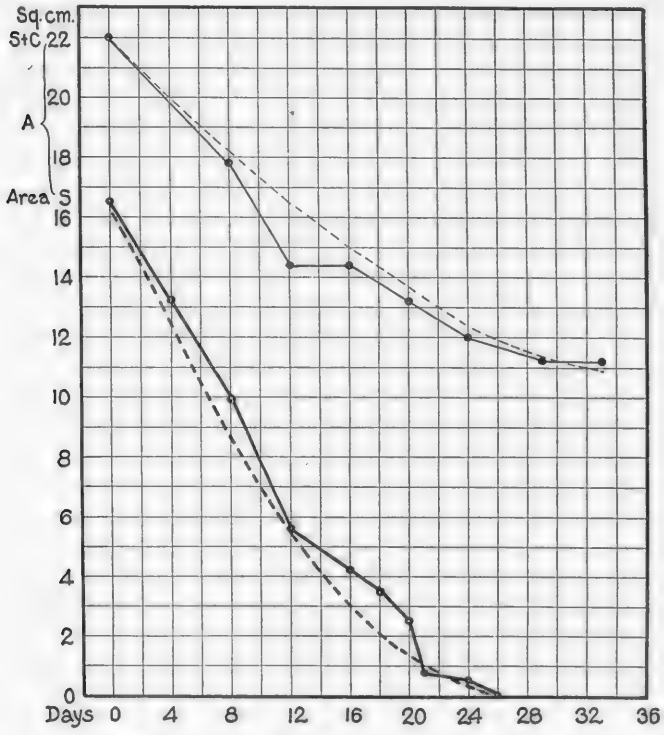
$$S_T = S_o e - K\left(T + \frac{T^2}{2p}\right)$$

ction.

of technique see

curve calculated

that this factor represents the epithelization. It must not be inferred that the latter remains proportional to $\frac{T^2}{2p}$ and increases at first slowly, then rapidly. On the contrary, we know that epitheliza-



TEXT-FIG. 5. The upper curves are the contraction curves. The dotted curve is calculated according to the formula

$$S_T = S_o e^{-KT}$$

The lower curves are the so called cicatrization curves expressing the decrease of the area of the granulations.

tion, or growth of cells, is likely to be much more active at the beginning of the cicatrization, according to the length of the epithelial edge, and then must decrease in absolute value. In proportion as the

wound decreases, the length of the epithelial edge diminishes, and at the same time the number which measures, in absolute value, the proliferation of cells. But, as it is likely that the number of cells produced by a unit of length is the same for each unit of time, and as, on the other hand, ordinarily the area decreases much faster than the perimeter (four times more rapidly for the square), it is clear that the production of cells by the edges seems to increase and that if it is expressed by units of covered area it increases really with reference to the area of the wound. What must therefore be understood by "the factor represents the epithelization," is that in the considered equation owing to the introduction of this factor, the relations existing between epithelization and the decrease of the area are satisfactorily expressed, and that it enables us to express the result of the phenomena in a way which is in accordance with the facts.

End of the Phenomenon.

Since in a logarithmic curve the diminution of the ordinate is always proportional to the ordinate, it never becomes zero. The curve, as well as that which had been established previously, is asymptotic to the axis of the time. But we have already stated, in a former paper, the moment at which cicatrization comes practically to an end.[4] This happens when our methods of measuring are unable to estimate the progress of the phenomenon. This moment is rapidly followed—in a few hours—by complete healing of the wound. The curve practically comes to an end, and experience has shown that it can be arbitrarily stopped, when the ordinate is inferior to 0.4 sq. cm. This means that, when the calculation comes to a figure smaller than 0.4 sq. cm., the corresponding abscissa, that is the time, indicates the date of complete cicatrization. Besides, this conforms to the facts in the majority of cases, as has been shown before, and the errors are small. In all natural phenomena, the law of which is expressed by an exponential equation, the same holds true.

Numerical Value of the Coefficients. Relation of K, the Index of Cicatrization i, and the Parameter 2p.

Calculation of fifteen cicatrization curves has shown principally three facts. The first, to which I referred above (page 334), is the pro-

[4] du Noüy, P. L., *J. Exp. Med.*, 1916, xxiv, 451; 1917, xxv, 721.

portional variations of K and of the index of cicatrization. Table II shows this plainly. The ratio $\frac{i}{K}$ varies between 1.6 and 1.2 inversely to i and K. The second fact is the remarkable constancy of the factor $2p$, or parameter of the parabolæ expressing the acceleration due to the epithelization. The third fact is the relation which seems

TABLE II.

Comparative Numerical Results.

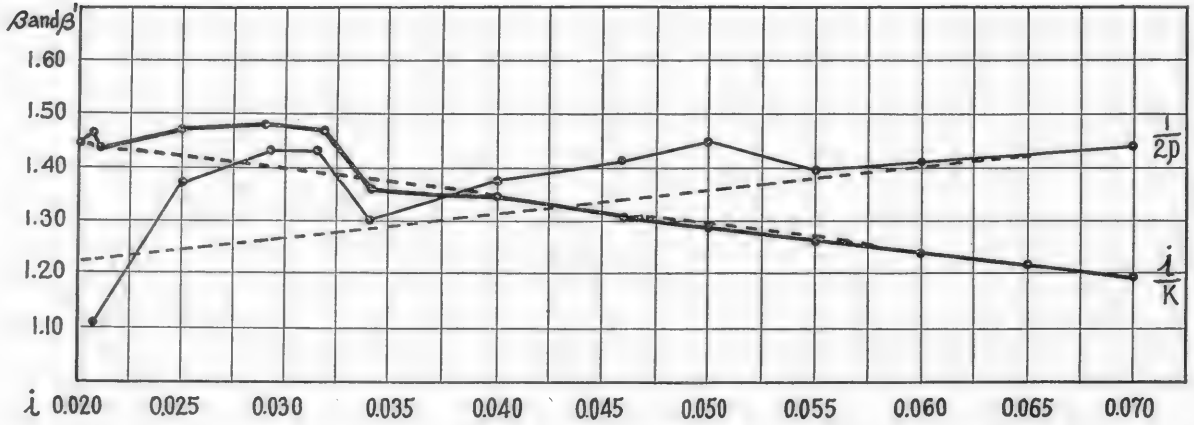
No. of patient.	Area.	Index i.	K	$2p$	$100\frac{K}{i} = \delta$	$\frac{1}{\beta'}$	$\frac{i}{K} = \beta$
	sq. cm.						
318	64.0	0.0200	0.0132	80	66		1.51
737	50.3	0.0200	0.0138	90	69	81.0	1.45
263	61.8	0.0205	0.0140	85	68	80.5	1.46
360	113.0	0.0210	0.0147	90	70	80.0	1.43
795	21.6	0.0255	0.0174	73	68.5	79.0	1.46
721	40.4	0.0285	0.0192	70	68	78.5	1.46
706	27.4	0.0325	0.0222	70	68.5	77.5	1.46
724	13.9	0.0346	0.0255	77	74	77.0	1.36
725	30.6	0.0375	0.0277	75	74	76.0	1.35
791	23.0	0.0400	0.0295	73	74	75.5	1.35
692	31.2	0.0420	0.0315	75	75	75.0	1.33
722	19.0	0.0465	0.0355	71	76	74.5	1.31
383	17.5	0.0500	0.0387	69	77	74.0	1.29
796	8.9	0.0550	0.0436	72	80	72.0	1.26
715	9.5	0.0700	0.0595	65	85	66.0	1.20

to exist between K, i, and $2p$. This is clearly shown by the seventh column in which is reported the term

$$\delta = 100\,\frac{K}{i}$$

The value of δ for each wound is near enough to $2p$ to allow its substitution for $2p$, approximately. This result is a natural conclusion of the first two remarks, since, if we call β the ratio $\frac{i}{K}$, we can write

$$2p = \frac{1}{\beta} = \frac{\delta}{100}$$



TEXT-FIG. 6. Relations between i, K, and $2p$. The ordinates represent $\frac{i}{K} = \beta$ and $\frac{1}{2p} = \beta'$. Light lines, β'. Heavy lines, β.

But this is of immediate value for calculating the curve by equation (9), because, if the coefficient K can be determined by giving two experimental dates 4 days apart, the same process cannot be used for determining the parameter $2p$, for the parabola

$$\alpha = \frac{T^2}{2p} \quad .$$

can only be determined when T is great enough; *viz.*, 12 or 16 days. Otherwise the ordinates α are smaller than 1 and the curve is not accurately defined. It is therefore worth while to be able to make an approximate calculation first. If this shows a noticeable error, it is easy to make a correction, as soon as several days have passed. Consequently, it is clear from the above paragraphs that it is possible to calculate the curve resulting from the equation

$$S_T = S_0 e^{-K\left(T+\frac{T^2}{2p}\right)} \tag{9}$$

by simply starting from a single measure of the wound and the age of the patient, that is from the index, since

$$K = \frac{i}{\beta}$$

Text-fig. 6 shows the relations between K and i. In order to make it clearer, I have plotted close to each point on the observed curve (dotted line) the inverse value of β; that is, $\frac{1}{\beta}$. The light curve which expresses the observed variations of $\frac{1}{2p} = \beta'$ shows that $2p$ varies approximately inversely to β since every point on the curves can be expressed by the inverse value of the ordinate. This means that $2p$ varies inversely to δ. If we admit as a possibility that the light dotted line corresponds to the average mean value of $2p$ and that the values which deviate from it are due to errors of calculation, the probable values of $2p$ can be computed for a certain value of i. The figures in the column marked $\frac{1}{\beta'}$ (Table II) may be used for the first approximation of $2p$.

Calculation of the Coefficients.

These analogies may be of use in determining $2p$, but sometimes a less accurate approximation is obtained when both K and $2p$ are inferred from the index. In such a case the direct calculation of K, which is extremely simple, is of more value. It is deduced, as stated above, from equation (9) which gives

$$K = \frac{1}{T} \operatorname{Log} \frac{S_o}{S_t}$$

S_o being the first measure of the area of the wound, S_t the area at the time t (practically 4 days). After K has been determined, at least two values of α must be calculated unless the relations between the coefficients, mentioned above, are employed. We have stated

$$\alpha = \frac{1}{K} \operatorname{Log} \frac{S_o}{S_T} - T \tag{7}$$

The values of S corresponding to 12 and 20 days, for instance, are taken. (The greater T is, the more accurate the values of $2p$ will be within the limits of 20 to 40 days.) $2p$ is immediately obtained by means of the formula

$$2p = \frac{T^2}{\alpha} \tag{8}$$

Since we have two values of α, we obtained two values of $2p$ and the mean value is taken.

The coefficient K is smaller than i and the quantity α must remain positive. If the contrary happens ($\alpha < 0$), a determination of K for a longer period of time (5, 6, or 8 days) must be made. This rarely happens.

Use of the Equation. Calculation of the Curves; Numerical Examples.

In order to enable the reader who is unfamiliar with the use of mathematical formulas, to use this equation, we shall make the complete calculation of one curve by using successively the direct calculation, or ordinary method, i being the supposed unknown, and then the method based upon the analogies existing between the coefficients. The difference in accuracy of both methods will thus be noted. Whenever the index i varies around 0.04 the results obtained by the second

technique are excellent. The reason is evident from Text-fig. 6 in which the values of the coefficients are in accordance with this particular value of i.

(*1*) *Direct Calculation.*—Only the initial area and that after 4, 8, or 20 days are given (Table III).

TABLE III.

Example of Direct Calculation.

T	S, observed area.	Log S	Log $\frac{S_o}{S}$	α	$2p$	Calculated area.
	sq. cm.					*sq. cm.*
0	40.4	1.602				
4	33.5	1.525	0.077	0		
8	27.0	1.431	0.171	0.9	71	27.0
20	12.5	1.097	0.505	6.2	65	12.8
28	6.8					7.0
36	3.5					3.5
44	1.7					1.7
54	0.6					0.6

The calculation requires accordingly (*a*) the determination of K

$$\left(K = \frac{\text{Log } S_o - \text{Log } S_T}{T}\right), \qquad K = \frac{0.077}{4} = 0.0192$$

(*b*) the determination of α (8th day)

$$\left(\alpha = \frac{\text{Log } S_o - \text{Log } S_T}{K} - T\right), \qquad \alpha = \frac{0.171}{0.0192} - 8 = 0.9$$

(*c*) the determination of $2p$ (8th and 20th days)

$$\left(2p = \frac{T^2}{\alpha}\right), \qquad 2p = \frac{64}{0.9} = 71$$

and finally the calculation of the points of the curve for the given times by formula (9)

$$\text{Log } S_T = \text{Log } S_o - K\left(T - \frac{T^2}{2p}\right)$$

(*2*) *Indirect Calculation.*—This is based only upon the relations previously stated between i, K, and $2p$. $2p$ may be taken either from

Table II or from Text-fig. 6, for the given value of i. In the preceding example $i = 0.0285$ (Table V). For this value, the table indicates $\frac{1}{\beta'} = 2p = 78.5$. The factor given by Text-fig. 6 is 1.41. Hence $K = \frac{i}{1.41} = 0.0202$. By applying the formula the areas given in Table IV are calculated (compare with Table III).

This shows that the values are as good as those obtained by the direct method, sometimes even better, because in the latter method $2p$ has only been determined from two points on the curve, which is a cause of error.

A direct determination of K can also be made and only the value of $2p$ read in the table. The results obtained by this intermediate technique are good, but it shows no particular advantage, and, on the contrary, introduces a new factor of error and requires more time.

TABLE IV.

Area (S).					
8th day.	20th day.	28th day.	36th day.	44th day.	54th day.
sq. cm.	*sq. cm.*	*sq. cm.*	*sq. cm.*	*sq. cm.*	*sq. cm.*
27.0	12.4	6.8	3.5	1.6	0.5

It must be borne in mind that the determination of a curve by a single measure of the area and the normal index presents many advantages which may be of greater interest than the perfect coincidence between two curves obtained by two equations of different form. The advantages are: (1) the possession of the normal curve of cicatrization corresponding to the normal index, characterizing the age of the patient; this curve is used as a standard with which the individual curve is compared, if they do not agree; (2) the elimination of errors due to two measures of the wound, because during the time elapsed between the measures—4 days for example—a slight acceleration or a slight lessening in the rate might have occurred. The so called indirect method, therefore, should be used when the normal curve of a wound is to be calculated. In order to facilitate this calculation, I have drawn Text-fig. 7 similar to Text-fig. 6, except that the ob-

TABLE V.

Calculation of the Curve of Cicatrization.

The Two Coefficients of the Formula

$$S' = S[1-i(t+\sqrt{nt})].$$

Area of wound.	1st coefficient—index of cicatrization i. Age of patient.					2nd coefficient (time coefficient) $t+\sqrt{nt}$.
	20 yrs.	25 yrs.	30 yrs.	32 yrs.	40 yrs.	
sq. cm.						
150 and over.	0.0200	0.0200	0.0200	0.0200	0.0200	6.00
						6.81
						7.43
140	0.0210	0.0200	0.0200	0.0200	0.0200	8.00
						8.45
130	0.0220	0.0200	0.0200	0.0200	0.0200	8.90
						9.30
120	0.0225	0.0200	0.0200	0.0200	0.0200	9.65
						10.00
110	0.0240	0.0200	0.0200	0.0200	0.0200	10.32
						10.64
100	0.0250	0.0200	0.0200	0.0200	0.0200	10.93
						11.21
90	0.0275	0.0220	0.0200	0.0200	0.0200	11.48
						11.75
80	0.0300	0.0230	0.0200	0.0200	0.0200	12.00
						12.25
70	0.0325	0.0250	0.0200	0.0200	0.0200	12.48
						12.72
60	0.0355	0.0300	0.0225	0.0200	0.0200	12.95
						13.16
50	0.0400	0.0340	0.0265	0.0230	0.0200	13.37
						13.60
40	0.0445	0.0400	0.0310	0.0270	0.0220	
30	0.0500	0.0450	0.0375	0.0330	0.0260	
25	0.0540	0.0500	0.0400	0.0375	0.0290	
20	0.0580	0.0540	0.0465	0.0425	0.0325	
15	0.0645	0.0600	0.0525	0.0475	0.0380	
10	0.0700	0.0660	0.0625	0.0550	0.0450	
5 and under.	0.0800	0.0750	0.0700	0.0700	0.0700	

served points are suppressed and the scale of the ordinates is larger, so that a greater accuracy may be obtained. To show the degree of approximation obtained by the new technique I have collected the calculations of four wounds. The figures calculated according to the extrapolation and exponential equations correspond to every observed area. These curves have been chosen intentionally, so that their indices are different. The calculation of the coefficients K and $2p$ is then simply done by looking for the index in Table V in function of the age of the man and of the area of the wound; then by using the relations (Table II)

$$K = \frac{i}{\beta} \text{ and } 2p = \frac{100}{\beta'}$$

calling β the observed values of $\frac{i}{K}$ (solid line) and β' the observed values of $\frac{1}{2p}$ (dotted and broken line), the values of $2p$ can also be found in Text-fig. 7, since the values of Table II have been calculated from this straight line. Text-fig. 7 is only used in order to give two conversion factors β and β', to be applied for computing K and $2p$.

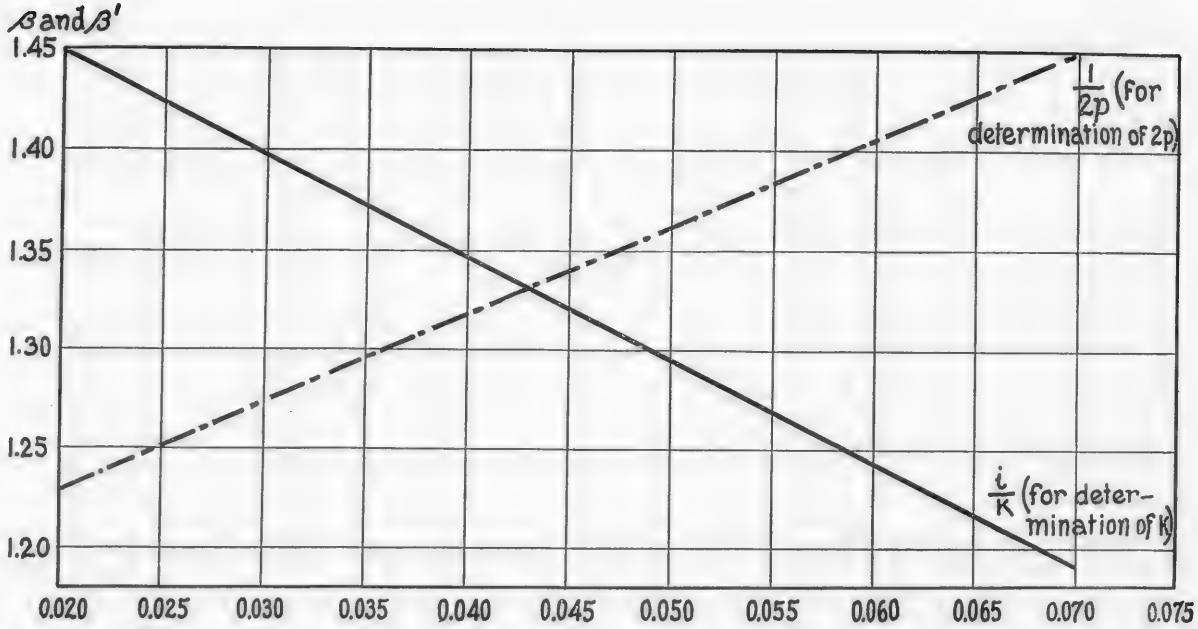
Comparative Examples.

Patient 360.

$$S_o = 129.4 \text{ sq. cm.}, i = 0.021, \beta = 1.44, \beta' = 1.23, K = \frac{i}{\beta} = 0.01458,$$

$$2p = \frac{1}{\beta'} = \frac{1}{1.23} \times 100 = 81.$$

Area.	8th day.	20th day.	44th day.	60th day.	76th day.	84th day.
	sq. cm.	*sq. cm.*	*sq. cm.*	*sq. cm.*	*sq. cm.*	*sq. cm.*
Observed	105.0	57.0	13.8	4.1	1.8	0.6
Calculated { Equation (1)	96.8	55.9	14.0	4.7	1.9	1.0
Calculated { " (9)	96.5	56.0	13.4	3.9	1.4	0.4



TEXT-FIG. 7. Relations between i, K, and $2p$. The chart gives the ratio $\beta = \frac{i}{K}$ and $\frac{1}{2p} = \beta'$, by means of which K and $2p$ can be calculated.

Patient 488.

$S_o = 34.5$ sq. cm., $i = 0.03$, $\beta = 1.40$, $\beta' = 1.27$, $K = 0.0214$, $2p = 79$.

Area.	8th day.	20th day.	32nd day.	44th day.	48th day.
	sq. cm.	*sq. cm.*	*sq. cm.*	*sq. cm.*	*sq. cm.*
Observed	20.6	11.0	4.0	1.7	0.9
Calculated { Equation (1)	22.4	9.9	3.7	1.3	0.8
Calculated { " (9)	22.2	10.0	3.8	1.2	0.7

Patient 694.

$S_o = 44.3$ sq. cm., $i = 0.0425$, $\beta = 1.33$, $\beta' = 1.33$.

Area.	8th day.	16th day.	20th day.	28th day.
	sq. cm.	*sq. cm.*	*sq. cm.*	*sq. cm.*
Observed	23.6	11.2	8.5	2.5
Calculated { Equation (1)	23.5	10.5	6.9	2.4
Calculated { " (9)	23.1	10.7	6.8	2.6

Patient 519.

$S_o = 19.0$ sq. cm., $i = 0.0570$, $\beta = 1.26$, $\beta' = 1.39$.

Area.	4th day.	12th day.	20th day.	28th day.
	sq. cm.	*sq. cm.*	*sq. cm.*	*sq. cm.*
Observed	12.2	4.2	1.0	0.4
Calculated { Equation (1)	12.5	4.4	1.2	0.3
Calculated { " (9)	12.2	4.4	1.3	0.3

CONCLUSION.

1. The law of cicatrization of surface wounds may be expressed by an exponential formula in which the two coefficients may be determined.

2. A simple relation exists between these coefficients and the index, i, of cicatrization, previously established in function of the age of the patient and of the area of the wound.

3. The proposed equation with a simplified exponent, reduced to a single coefficient, expresses satisfactorily the phenomenon of contraction.

OBSERVATIONS ON THE WASSERMANN REACTION.

A Comparison of the New System of Noguchi with That Using Cholesterolized Antigen According to McIntosh and Fildes.

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Among the many modifications of the Wassermann reaction, that of Noguchi,[1] introduced a number of years ago, in which use is made of the acetone-insoluble lipoids extracted from certain tissues and the anti-human hemolytic system, is doubtless the most promising from a theoretical standpoint. In practice we had found it a troublesome system to apply particularly because the collection of the human cells for the immunization of rabbits and carrying out the tests is laborious and time-consuming, and also because the production of the anti-human amboceptor in the rabbit is less satisfactory than that of anti-sheep amboceptor. We had come to the conclusion that if a certain allowance was made for reactive anti-sheep amboceptor, as can be done by an intermediate incubation period of 15 to 20 minutes after adding the sheep cells, but before adding the anti-sheep amboceptor, the results did not differ materially from the human hemolytic system. For a long time we used the Noguchi antigen with satisfaction.

McIntosh and Fildes[2] presented an application of a principle introduced by Sachs (the addition of cholesterol to alcoholic tissue extracts), and Richardson[3] compared the reactions with this "choles-

[1] Noguchi, H., Serum diagnosis of syphilis, Philadelphia, 1910.

[2] McIntosh, J., and Fildes, P., An investigation of the value of certain antigens for use in the Wassermann reaction, in particular of Sach's new antigen, *Z. Chemotherap., Orig.*, 1912–13, i, 79.

[3] Unpublished observations at the Pennsylvania Hospital.

terolized antigen" with those obtained with the Noguchi antigen with the anti-sheep hemolytic system. He studied about 2,000 reactions, and, like other investigators, found that the cholesterolized antigen gave the higher percentage of positive reactions, but that in most cases this was justified by the facts. Eight cases were found in which there was no evidence of syphilis other than the positive reaction, but there was no evidence to the contrary and it was considered that the result was on the whole satisfactory. This antigen is the easiest to obtain in quantity and is of relatively constant quality as compared with others. It was therefore adopted and has been used continuously for a number of years in these laboratories. More recently the carrying out of the reactions has passed into other hands, and we have had some reason to question the accuracy of the opinion previously held as to the safety of the cholesterolized antigen from the diagnostic point of view.

In the meantime, opinion elsewhere seems to be divided. Some competent observers have found the cholesterolized preparation satisfactory. In the hands of others it seems to be too sensitive; that is, it gives rise to false positives.

Recently Noguchi[4,5] has proposed the use of the native human complement in the reaction with his acetone-insoluble lipoids as antigen. He states that with the quantities he specifies satisfactory results can be obtained, fully comparable with those derived from the use of his older system. This system makes unnecessary the continued collection of guinea pig serum, and this may be expected to compensate for the trouble in regard to human red cells. It is, of course, of importance at times to be able to carry out the method when guinea pig serum may not be available.

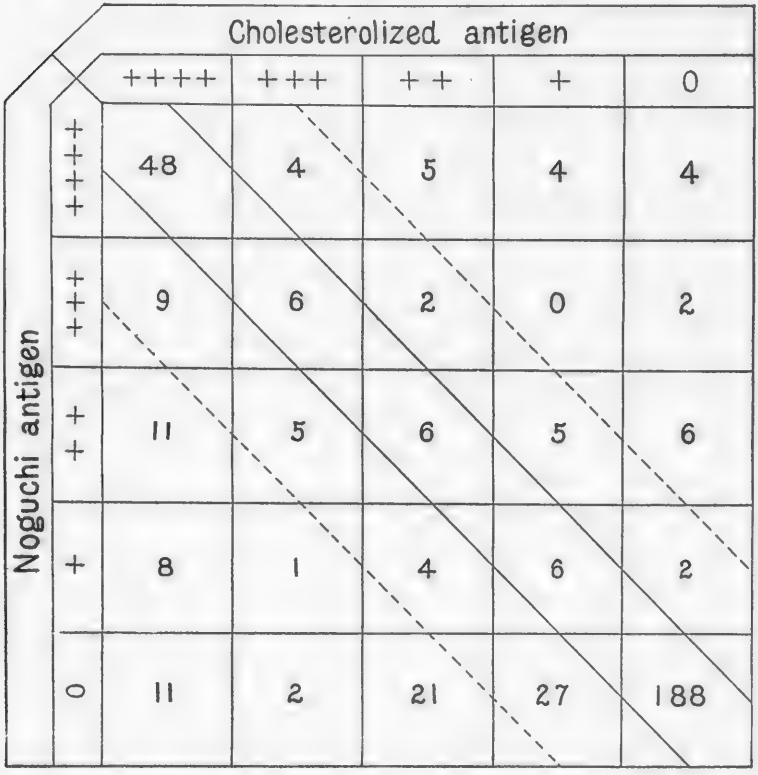
We have attempted, therefore, to review the question of the Wassermann reaction as practically applied with the purposes of (1) reconsidering the suitability of the cholesterolized antigen, and (2) examining the merits of the newer Noguchi system.

[4] Noguchi, H., A method of facilitating the serum diagnosis of syphilis under war conditions, *J. Am. Med. Assn.*, 1918, lxx, 1157.

[5] Noguchi, H., A homohemolytic system for the serum diagnosis of syphilis, *J. Exp. Med.*, 1918, xxviii, 43.

RESULTS.

It has been assumed on the basis of the author's report that the two systems of Noguchi would have equal value and that comparison of one of them with the results by cholesterolized antigen should



TEXT-FIG. 1. The figures in the chart indicate the number of sera giving deviations as indicated with both the cholesterolized antigen and according to Noguchi's homohemolytic system. The total comprises 387 different sera.

establish the place of the latter. We have therefore done our reactions comparing the newer Noguchi system with the system which makes use of cholesterolized antigen and the anti-sheep hemolytic reagents. The general results are displayed in Text-fig. 1.

It is apparent that in a large majority of the cases the two methods of work have an equal value (central diagonal space) or one essentially equal (adjacent diagonal spaces). It is also clear that the numbers toward the lower left corner (cholesterol reaction positive, Noguchi reaction negative) considerably outnumber those toward the upper right (cholesterol reaction negative, Noguchi reaction positive). In other words, the cholesterolized antigen is considerably more reactive.

Which method develops the facts most nearly in accord with the truth as to the existence of syphilis in the particular cases? Of the ten cases in which the Noguchi system gave a positive and the other a negative or weak reaction, eight were unquestionably syphilitic. One of the others was possibly syphilitic. In the tenth case, that of an apparently healthy young man with a fractured jaw, no decisive evidence could be obtained. In all these cases except one, who was an undoubted syphilitic, doubling the amount of serum used (making 0.2 cc.) in the reaction with the cholesterolized antigen perceptibly increased the intensity and in most instances rendered it entirely positive.

Material.

Before taking up the question of whether a positive reaction with the cholesterolized antigen means syphilis, even though the Noguchi reaction be weak or absent, it is perhaps best to consider the material which we have had for study. The majority of our reactions were carried out on known cases of syphilis, who were under treatment. In most of these the evidence of disease was clear apart from the Wassermann reaction. Most of the cases which showed a positive cholesterol and negative Noguchi reaction come in this group. As controls we examined the blood of twenty-nine persons in good health who were sufficiently known to us to make it appear improbable that they were suffering from syphilis. One of these gave +++ reaction with cholesterolized antigen and on the same occasion gave a ++ Noguchi reaction. On another occasion the same person gave a ++ cholesterol and a negative Noguchi. Two others in the group gave a ++ cholesterol reaction and a negative Noguchi. Further, the blood of 109 patients at one time available in the hos-

pital was examined without selection. Whether by chance or not these results, which will be considered in detail later, do not bear on the present point as the two reactions were practically alike in all instances.

Of the cases in which the cholesterol reaction was positive (++++ or +++), the Noguchi reaction being at the same time weak or negative, five could not be satisfactorily determined although there is no reason to believe that the reaction is in error. The remainder were definitely syphilitic and there is every reason to believe that the reaction with cholesterolized antigen is a better expression of their condition than that done by the Noguchi method.

There are twenty-one reactions showing a ++ cholesterol with a negative Noguchi reaction. In this series these reactions are for the most part the evidence of a fading out of the activity of the serum in the course of specific treatment. In view of the three reactions of similar intensity among twenty-nine probably normal individuals such degrees of activity cannot, of course, be held to be of any decisive value from a diagnostic view-point. Whether the six instances in which the Noguchi reaction was ++ and the cholesterol reaction negative are similarly lacking in diagnostic significance may be open to question, but we are inclined to the belief that they are, except, as may sometimes happen, when doubling the amount of test serum leads to a stronger reaction with cholesterol.

Interpretation of Results.

In view of the results in the unselected series of patients in the hospital, it appears to be useless to discuss the question of just which intensity of reaction, with either antigen or both, justified a diagnosis of syphilis. Among these 109 persons there were several cases in whom the diagnosis of syphilis was presumptive on other grounds. These gave clear-cut reactions with both systems. There were two patients who gave ++++ Noguchi reactions with weak cholesterol reactions. The status of these cases remains in doubt. In addition, the cases shown in Table I gave more or less reaction.

These cases comprise about half the febrile cases in the hospital at the time. The others, rheumatic fever, endocarditis, and lobar pneumonia, as well as about 50 cases in the surgical wards, gave

negative reactions. It is evident, therefore, that the Wassermann reaction must be used with some caution and that it must always be valued in conjunction with a presumptive diagnosis based on other grounds. With these facts in mind it may be fairly stated that from the point of view of establishing a diagnosis the two forms of reaction have approximately equal value. The cholesterol reaction is somewhat more sensitive and the weaker grades are to be viewed with a little less confidence perhaps than similar weak reactions with the Noguchi system. Whenever possible there would

TABLE I.

Case No.	Diagnosis.	Cholesterol reaction.	Noguchi reaction.
13	Typhoid fever	+++	++++
16	" "	++	0
18	" "	++++	+++
20	Heat stroke	+	0
24	Typhoid fever	++++	++
25	" "	++++	++
26	Heat stroke	++++	++++
28	Typhoid fever	+	0
29	Heat stroke	+	0
30	Lobar pneumonia	+++	+
45	Gastroenteritis	0	++
54	Asthma	0	++
57	Tuberculosis; peritonitis	++	0
58	Acute enteritis	0	++

appear to be a real advantage in viewing the two systems side by side, holding for particularly careful scrutiny cases in which the results do not check.

Fully as important as its application to diagnosis is the use of the Wassermann reaction as a control for treatment. Here any difference that may exist in sensitivity is in favor of the continued use of the cholesterolized antigen. It may also be profitable to use the two systems side by side. One purpose of this would again be that of having a precautionary check. There is imperfect evidence in our hands, however, that under treatment the two systems may vary in some degree independently of one another, and it is possible that useful information may be obtained by following out this suggestion.

Up to this point we have spoken of these two types of reaction as though the results with either were always unmistakable and arrived at without difficulty. This is by no means the case. Exception has frequently been taken to the use of cholesterolized antigen because of irregularities which develop with successive reactions with the same samples of serum. In order to make the reaction safe it seems to us necessary to use a slight excess over the amount of complement usually advised for Wassermann work. Our custom is to use $2\frac{1}{2}$ units of both complement and amboceptor. The final antigen dilution should be used within a few minutes after it is made and should be made in accordance with McIntosh and Fildes' directions; that is, the mixture with salt solution should be rapidly accomplished. We put the salt solution (24 cc.) in an Erlenmeyer flask of 50 cc. capacity, float the alcoholic solution very carefully on the surface, and then mix the two with one quick vigorous shake. The titration of reagents is carried out completely each time on the basis of $\frac{1}{2}$ hour incubation in the water bath and the incubation after the addition of the hemolytic system is likewise $\frac{1}{2}$ hour.

Neither is the Noguchi system in its present form free from difficulty. It should be noted that in the two publications on the subject Noguchi has recommended the use of two different quantities of human serum. He first suggested 0.1 cc. as the proper quantity, later changing this to 0.2 cc. in his final paper. Neither recommendation appears to us to be adequate. As a matter of actual observation there are great differences in the susceptibility of various samples of human red corpuscles to hemolysis by human complement and rabbit anti-human amboceptor. These differences are quantitatively such that a given serum may present insufficient complement for the reaction or may have more than four units. From 70 consecutive tests we found that with susceptible corpuscles every fresh human serum has complement sufficient for the test. Certain sera with these corpuscles may contain as much as eight units of complement. With resistant corpuscles no serum may have more than two units and one in ten may show no complement whatever. An intermediate condition is more common and was evidently encountered by Noguchi. If exact work is to be done account must be taken of these facts.

We have found it best to conduct each test with three quantities of serum, 0.2, 0.1, and 0.05 cc., each quantity having its antigen and control tubes. The result is then scarcely ever in doubt. It would doubtless be still better to standardize the reaction to a certain extent by selecting a single suitable person, preferably one with susceptible corpuscles, as a source of the red cells.[6] If this were done it would probably be safe to rely on two of the above quantities of serum, either the greater or the lesser pair as the susceptibility of the cells indicated.

Another objection to the system which at first sight seems important is negligible in practice. It is obviously impossible to control the anticomplementary value of the antigen for each separate complement where the complement is furnished by the test serum. The antigen which we have been using has had an apparent anticomplementary value of about ++ on the unit value of complement and this can be allowed throughout the tests whenever deviation occurs. For each lot of antigen this would necessarily be determined by comparative observation on a number of negative sera. Noguchi speaks of his antigen's being entirely without anticomplementary value in the quantity recommended. In this particular our application of the method can doubtless be somewhat improved upon.

SUMMARY.

The Wassermann reaction carried out according to the method of McIntosh and Fildes, with cholesterolized antigen and with certain allowances for the presence of native anti-sheep amboceptor, leads to about the same result as when it is done according to the recent proposal of Noguchi, with the native human complement and acetone-insoluble lipoids as antigen. The differences are such as to suggest that from the point of view of diagnosis the Noguchi method is the more conservative but that there is definite advan-

[6] Observations which have been begun but are as yet unfinished indicate that the source of variation in the resistance of the corpuscles may be sought rather in the way the cells are kept or their age outside the body than in any true variation from person to person. The outcome of this study in no way influences the practical significance of the considerations put forward above.

tage in using two methods as distinct in origin of materials as these, partly for the purpose of control and partly in the hope of acquiring new information of importance. As a measure of control of treatment the cholesterol antigen appears to us to be the more valuable. The Wassermann reaction alone, by whatever method it may be done, can only be used in the diagnosis of syphilis in conjunction with presumption based on other grounds. That it fails to appear in a considerable percentage of syphilitics is well known. That the reaction is positive in other conditions is not so generally recognized. Fresh instances of this in certain febrile cases are here recorded.

MIGRATION OF PARASITES AS THE CAUSE OF ANEMIA IN ÆSTIVO-AUTUMNAL MALARIAL INFECTIONS.*

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(*From the Laboratory of Dr. Mary R. Lawson, New London.*)

Plates 17 to 19.

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Malarial Anemia.

Malarial infections are characterized by the early development of a more or less severe anemia. As a rule, the æstivo-autumnal infections present the gravest manifestations. The severity of the anemia and its rapid appearance have frequently been noted.

Rossoni[1] states that no acute infection results in so active a deglobulization as does malarial fever, and that in all cases there is an immediate diminution in the number of corpuscles and the amount of hemoglobin. Manson[2] writes: "We find a degree of oligocythæmia greatly in excess of anything we might expect, or which can be accounted for by, or in correspondence with, the proportion of corpuscles attacked and directly consumed by the plasmodium."

Loss of Red Corpuscles in the Intervals between the Paroxysms.

Each parasite destroys several red corpuscles in the course of its development, the result of which is not only the loss of corpuscles with each paroxysm, but a loss in the intervals between paroxysms. That this interval loss has been noted might be understood from the following observations.

* Aided by a grant from The Rockefeller Institute for Medical Research.

[1] Rossoni, quoted from Thayer, W. S., and Hewetson, J., *Johns Hopkins Hosp. Rep.*, 1895, v, 60.

[2] Manson, P., Tropical diseases, a manual of the diseases of warm climates, London, 3rd edition, 1903, 88.

Thayer and Hewetson[3] state that in pernicious cases the number of red corpuscles may fall between the paroxysms. Marchiafava and Bignami[4] observed that anemia is produced in cases of pernicious fever which are not accompanied by elevations of temperature, the so called larval or masked pernicious fevers. They state: "Thus a patient of vigorous constitution in the first four days of a quotidian fever, or a remittent fever of first invasion, may show a reduction to 2,000,000 red blood corpuscles, or there may even be a reduction of 1,000,000 per cubic millimetre within twenty-four hours." Rossoni[5] states that the loss in hemoglobin and corpuscles is rarely evident during the paroxysm, but begins with apyrexia and may continue for several days afterwards. Dionisi[6] in a case of æstivo-autumnal infection observed a reduction of 500,000 red corpuscles in 12 hours, and he noted a fall from 3,200,000 to 2,300,000 corpuscles during the first 6 days of apyrexia in spite of the use of iron. Kelsch[7] saw a diminution of red corpuscles as low as 500,000 per c.mm. This diminution occurs, according to him, irregularly. Ewing[8] noted that the anemia may progress in the afebrile periods.

I have observed a continued loss of red corpuscles in the intervals between paroxysms.

Theories Advanced to Account for an Anemia Out of Proportion to the Number of Parasites Present.

The belief has been general that the only actual destruction of red corpuscles by parasites occurred when the parasites segmented, the destruction of the corpuscles corresponding to the segmentation of the parasites. Therefore when an anemia occurred out of proportion to the number of parasites present, provided each parasite destroyed but one corpuscle, certain theories were formulated to account for it.

[3] Thayer, W. S., and Hewetson, J., *Johns Hopkins Hosp. Rep.*, 1895, v, 57.

[4] Marchiafava, E., and Bignami, A., in Stedman, T. L., Twentieth century practice, New York, 1900, xix, 187, 188.

[5] Rossoni, quoted from Thayer, W. S., and Hewetson, J., *Johns Hopkins Hosp. Rep.*, 1895, v, 61.

[6] Dionisi, quoted from Ewing, J., Clinical pathology of the blood, New York, 1903, 2nd edition, 459, 460.

[7] Kelsch, quoted from Mannaberg, J., The malarial parasites. A description based upon observations made by the author and by other observers, translation by Felkin, R. W., London, 1894, 384.

[8] Ewing, J., *J. Exp. Med.*, 1900–01, v, 460.

Mannaberg[9] states: "It is possible that damage to the non-infested blood corpuscles is due to the parasitic poison which is dissolved in the *liquor sanguinis*. So we see that the red blood corpuscle in malarial patients may be attacked in two ways, but their destruction is far more due to direct invasion by the parasites than to the dissolved poison." Marchiafava and Bignami[10] write: "Nothing, however, forbids a belief that a special toxin of parasitic origin causes destruction of the red corpuscles by a specific action of its own. . . . . although the researches so far pursued with the purpose of directly proving the existence of poisons caused by the parasites have given negative results." Thayer[11] states "that there may well be other substances present in the circulation which result in the destruction of a number of non-parasitiferous elements." Ewing[12] writes: "Many factors indicate that the post critical anæmia is principally referable to globucidal action of the serum dependent upon the presence of a malarial toxin," one of the facts in support of this view being the "disproportion between the anæmia and the number of parasites present." Viola[13] believes that the destruction of red corpuscles depends only in a minor degree upon the direct action of the parasites and to a greater extent upon their toxic products.

As far as I can ascertain there has been no convincing evidence in support of the above views.

The Hemoglobin in Malarial Infections.

Reduction of hemoglobin out of proportion to the loss of red corpuscles is frequently observed. This reduction is easily explained. Two facts should make it clear: (1) a total destruction of red corpuscles accompanied by (2) a partial destruction by the parasites. There are at all times a certain number of parasites attached to corpuscles which are partially decolorized by parasitic action. When a red count and hemoglobin estimate are taken, each partially decolorized corpuscle counts as a corpuscle, and there is a partial loss of hemoglobin plus the total loss.

[9] Mannaberg, J., The malarial parasites. A description based upon observations made by the author and by other observers, translation by Felkin, R. W., London, 1894, 384.

[10] Marchiafava, E., and Bignami, A., in Stedman, T. L., Twentieth century practice, New York, 1900, xix, 199.

[11] Thayer, W. S., Lectures on the malarial fevers, New York, 1897, 185.

[12] Ewing, J., Clinical pathology of the blood, New York, 1903, 2nd edition, 461.

[13] Viola, quoted from Marchiafava, E., and Bignami, A., in Stedman, T. L., Twentieth century practice, New York, 1900, xix, 193.

Migration of Parasites in Æstivo-Autumnal Infections (Figs. 1 to 93).

It is practically impossible to ascertain the total number of migrations in the æstivo-autumnal infections as so few of the developmental stages are present in the blood. Migration of parasites is undoubtedly going on in the internal organs with consequent loss of red corpuscles. As stated above, each parasite destroys more than one red corpuscle; that is, after destroying one corpuscle, the parasite migrates to another and proceeds to destroy that. In the intervals between attachments the parasite is, for brief periods of time, free in the blood serum. All the parasites of one brood do not migrate at one time any more than do all the parasites of one brood segment at once.

In films containing free parasites in migration (Figs. 1 to 30, 35 to 64, and 66 to 69) one finds, usually, other parasites in similar stages of development, attached to dehemoglobinized red corpuscles (Figs. 65 and 86 to 93) or to corpuscular skeletons (Figs. 31 to 34) as well as attached to red corpuscles whose hemoglobin appears to be intact (Figs. 70 to 84).

The above stages of migration, when seen in one film, indicate that the process of migration is rapid and that the parasites do not long remain free. One would naturally expect an immediate attachment of the parasites when the surroundings are favorable to their growth, and no quinine or other injurious drug is present. I have seen parasites attaching themselves to fresh corpuscles before they had entirely abandoned the dehemoglobinized corpuscles.

Mannaberg[14] observed a tertian parasite (not a flagellating body) abandon a corpuscle, and he states: "It occurs with such rapidity and in such an unexpected manner that the details cannot be appreciated." In 1899, Dock[15] described what I believe to be migrating parasites. In several instances he saw free parasites. He pictures eight changes of form in a small, free, hyaline body, the observation lasting 5 minutes. He states that certain parasites seemed to have an especial tendency to escape from the blood corpuscles at early periods. And he seemed to be in doubt as to whether the free parasites were varieties of

[14] Mannaberg, J., The malarial parasites. A description based upon observations made by the author and by other observers, translation by Felkin, R. W., London, 1894, 344.

[15] Dock, G., *Med. News*, 1890, lvii, 61, 63.

the forms which carry on the typical process in malaria, or whether they had an independent existence. Bignami and Bastianelli[16] believed that, in the presence of a large number of free parasites, they had found evidence of an early destruction by decolorization of a large number of parasitiferous corpuscles. In 1914, Macfie,[17] in a case of æstivo-autumnal infection, observed an undoubted migration. He reports that a considerable number of free parasites were seen, many of which appeared to be young parasites which had not yet attached themselves. Other parasites, he states, were definitely ring-shaped, not infrequently possessing two chromatin masses. Undoubtedly the ring-shaped parasites and the parasites with the two chromatin masses had been recently attached to corpuscles and were freed in the form which they assumed when attached. The parasites with the two chromatin masses were two parasites, which had been attached to one corpuscular mound.

In studying migrations it will save time to work with the heavy infections.

Free Parasites.

Free parasites may be found in the blood of all malarial infections. In the æstivo-autumnal infections one may find free in the blood, round bodies and crescents, and parasites in the early stages of development. Instances of multiple infection of red corpuscles are more frequently seen in æstivo-autumnal infections than in other malarial infections. Where more than one parasite is attached to a corpuscle, they are set free prematurely by destroying the infected corpuscle sooner than one parasite would.

Free parasites may be (*a*) compact, (*b*) ring-form, or (*c*) ameboid, and they may show delicate filaments arising from the cytoplasm of the parasites for the purpose of attachment to fresh red corpuscles. Sometimes these filaments are so delicate as to be scarcely discernible and one's attention is first attracted to a pigment granule in connection with a filament so fine that otherwise it would not have been noticed.

Almost every worker who has done much research in connection with malarial parasites has seen parasites free, either in fresh or stained specimens. They have been so frequently seen and described that it seems surprising that a true interpretation of their presence in the blood did not suggest itself.

[16] Bignami, A., and Bastianelli, D. G., quoted from Thayer, W. S., Lectures on the malarial fevers, New York, 1897, 168.

[17] Macfie, J. W. S., *Lancet*, 1914, ii, 1354.

Laveran[18] writes of the parasites as "now free in the blood, now adherent to blood corpuscles." Mannaberg[19] has observed free not only "spores," but "parasites which are developed further than their spore-forming stage," and he states that the way their structure takes the stain is so striking that one is prevented from suspecting them to be degeneration forms, or from mistaking them for other blood elements. Canalis[20] has seen free parasites in æstivo-autumnal infections. Thayer[21] describes parasites as free in the cerebral capillaries. Marchiafava and Bignami[22] have observed free parasites. DaCosta[23] describes young parasites as escaping prematurely from red corpuscles. Ewing[24] states that, associated with intracellular parasites, he frequently saw young æstivo-autumnal parasites free in the plasma. Celli and Guarnieri[25] sketched the appearance of young extracellular bodies, including forms of at least two species of parasite. Gautier and Ziemann[26] have observed free parasites. Councilman and Abbott[27] described pigment-bearing bodies in and outside red corpuscles. Golgi[28] states that sometimes parasites may be seen free in the plasma. Ewing[29] must have attached a certain importance to the fact that free parasites were seen in the blood, for he has written of them under the heading of "Extracellular parasites."

The parasites illustrated in this article were secured from two cases of æstivo-autumnal infection.

[18] Laveran, A., Plaudism, translation by Martin, J. W., London, 1893, 12.

[19] Mannaberg, J., The malarial parasites. A description based upon observations made by the author and by other observers, translation by Felkin, R. W., London, 1894, 277.

[20] Canalis, P., quoted from Thayer, W. S., and Hewetson, J., *Johns Hopkins Hosp. Rep.*, 1895, v, 22.

[21] Thayer, W. S., Lectures on the malarial fevers, New York, 1897, 61.

[22] Marchiafava, E., and Bignami, A., in Stedman, T. L., Twentieth century practice, New York, 1900, xix, 291.

[23] DaCosta, J. C., Clinical hæmatology, Philadelphia, 1901, 365.

[24] Ewing, J., *J. Exp. Med.*, 1900–01, v, 446.

[25] Celli and Guarnieri, quoted from Ewing, J., *J. Exp. Med.*, 1900–01, v, 473.

[26] Gautier and Ziemann, quoted from Ewing, J., *J. Exp. Med.*, 1900–01, v, 473.

[27] Councilman and Abbott, quoted from Thayer, W. S., and Hewetson, J., *Johns Hopkins Hosp. Rep.*, 1895, v, 12.

[28] Golgi, quoted from Thayer, W. S., and Hewetson, J., *Johns Hopkins Hosp. Rep.*, 1895, v, 16.

[29] Ewing, J., *J. Exp. Med.*, 1900–01, v, 472.

SUMMARY.

The anemia in malarial infections is explained by the fact that each parasite destroys several red corpuscles.

Reduction of hemoglobin out of proportion to the loss of red corpuscles is explained by the fact that there is always a partial loss of hemoglobin in certain of the surviving corpuscles due to parasitic action.

Migration of parasites occurs in all æstivo-autumnal infections. If one wishes to observe migrations, it will save time to study the heavy infections.

Free parasites have been frequently noted by many observers, who have failed to interpret properly their presence in the blood.

EXPLANATION OF PLATES.

PLATE 17.

ÆSTIVO-AUTUMNAL PARASITES.

Magnification, × 1,780.

FIGS. 1 to 30. Free parasites in migration. The pigment granules (at o) are evidence of previous attachments.

FIGS. 31 to 34. Young parasites on corpuscular skeletons. In Fig. 34 a pigment granule is shown at o.

PLATE 18.

ÆSTIVO-AUTUMNAL PARASITES.

Magnification, × 1,780.

FIGS. 35 to 37. Free parasites with attaching pseudopodia arising from the cytoplasm of the parasites. Pigment granules (at o) are evidence of previous attachments.

FIG. 38. Three young parasites freed from one corpuscle.

FIGS. 39 to 41. Young parasites with attaching pseudopodia. Pigment granules indicating previous attachments may be seen at o. In Fig. 41 the filament connecting the pigment granule with the parasite is scarcely discernible.

FIGS. 42 and 43. Instances of two young parasites freed from one corpuscle. In Fig. 42 a pigment granule is seen at o.

FIGS. 44 to 58. Free ameboid young parasites. Pigment granules at o. Fig. 57 shows two free parasites; chromatin masses at x.

FIG. 59. Two free parasites; one is ameboid, the other at x is compact.

FIGS. 60 to 63. Instances of two young parasites freed from one red corpuscle. Note that at o pigment granules may be seen in connection with the parasites.

Figs. 60, 62, and 63 show pigment granules in connection with but one of the parasites. In Fig. 63 the parasite at x is compact.

FIG. 64. Three young parasites probably freed from one red corpuscle. The two at x are compact.

PLATE 19.

ÆSTIVO-AUTUMNAL PARASITES.

Magnification, × 1,780.

FIG. 65. A young parasite attached to a red corpuscle almost entirely dehemoglobinized, and an older parasite attached to a peripheral mound of a healthy appearing corpuscle. At x a young parasite is seen attached to a decolorized corpuscle.

FIGS. 66 to 69. These figures show young parasites free and also instances of two parasites attached to one corpuscular mound (at x). Fig. 67 shows two pigment granules at o.

FIGS. 70 to 79. Young parasites attached to fairly healthy appearing red corpuscles, the bodies of the parasites resting on the periphery of the corpuscle. Fig. 74 shows a pseudopodium arising from the cytoplasm of the parasite.

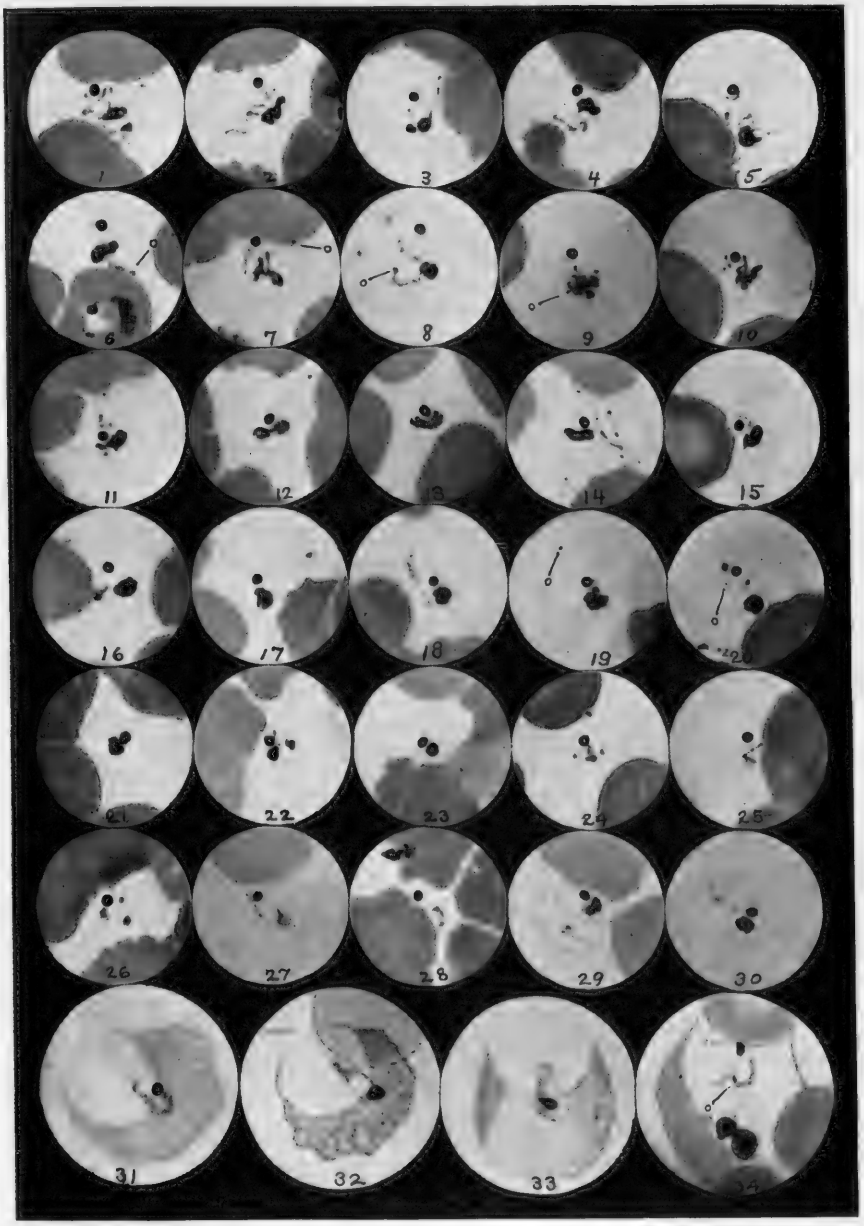
FIGS. 80 to 84. Young parasites attached to peripheral corpuscular mounds of healthy appearing red corpuscles.

FIG. 85. A young parasite attached to a corpuscle by a delicate pseudopodium.

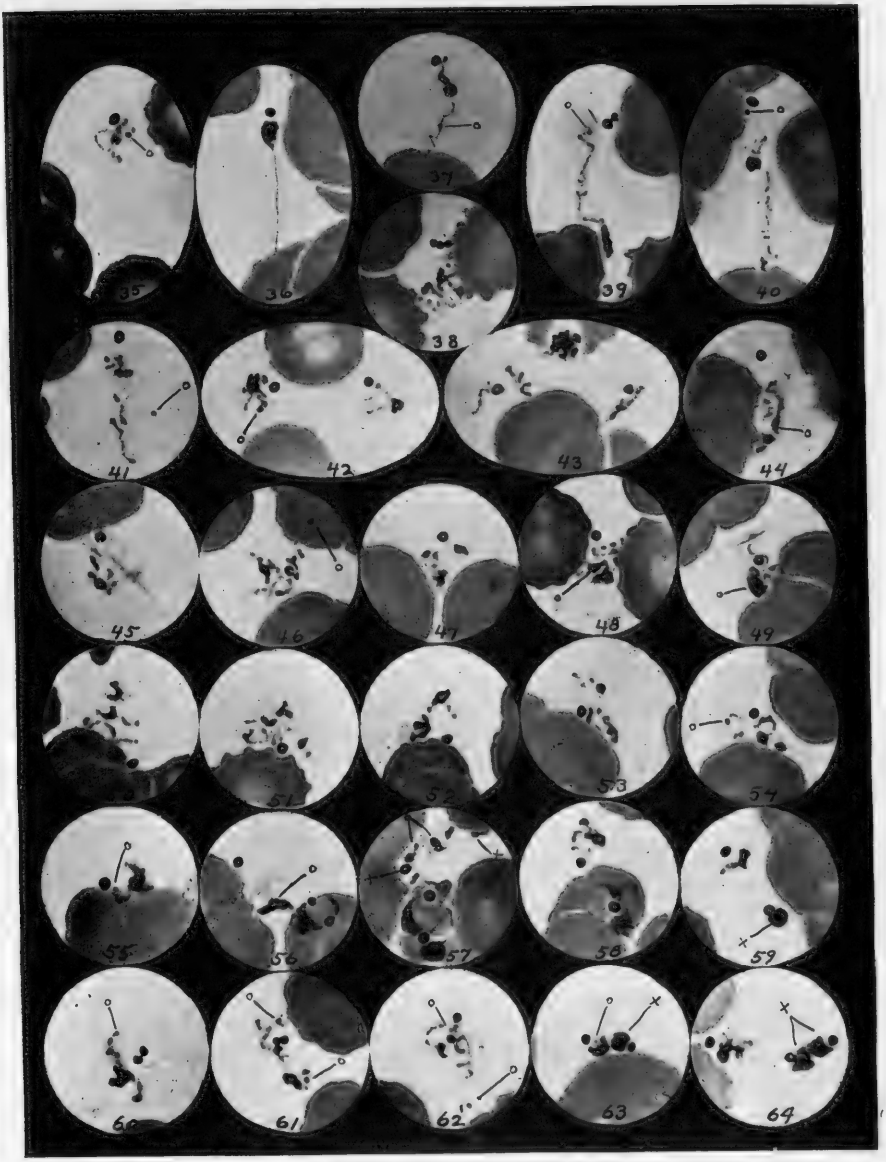
FIGS. 86 to 91. Parasites partly off decolorized red corpuscles. Pigment granules may be seen at o. In Fig. 89 a compact parasite is seen at x. In Fig. 91 two young parasites are seen attached to a healthy appearing corpuscle, while one parasite has decolorized the corpuscle at their right.

FIG. 92. A young parasite attached to a peripheral mound of a decolorized red corpuscle. Part of the parasite extends beyond the periphery of the corpuscle. A pigment granule is seen at o.

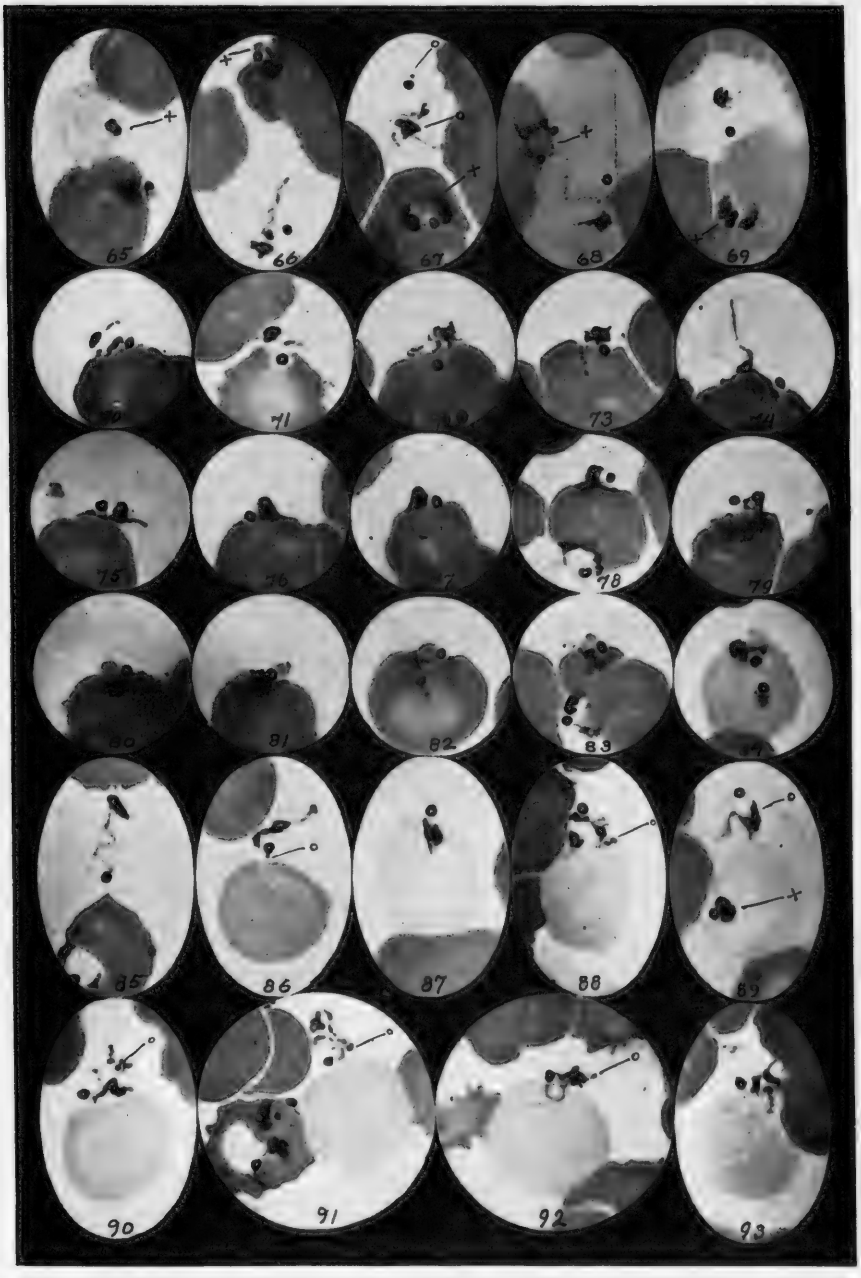
FIG. 93. A young parasite attached to a decolorized corpuscle.



(Lawson: Æstivo-autumnal malarial infections.)



(Lawson: Æstivo-autumnal malarial infections.)



(Lawson: Æstivo-autumnal malarial infections.)

THE FUNCTIONAL VALUE OF NEWLY FORMED CONNECTIVE TISSUE.

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Experimental tuberculosis, viewed broadly, presents a characteristic picture not to be confused with any other except that of the spontaneous or natural disease. Experiments devised to analyze the picture into its component parts have usually failed to develop information of decisive value. If, for example, parallel inoculations are made with living tubercle bacilli and with those killed by heat, the lesions produced are much alike in the beginning and remain so for some time. Finally, those due to the living bacilli are progressively reduplicated by the further invasion of the tissues, while those due to the dead bacteria are gradually healed. It is difficult again to say in what essential particular the lesions due to dead tubercle bacilli differ from those caused by other mildly irritant foreign bodies introduced into the tissues.

The lesions above mentioned have in common an initial acute inflammatory reaction and a subsequent deposit of more or less perfectly formed fibrous connective tissue. At the point of divergence this connective tissue in the case of the living tubercle bacillus undergoes degenerative changes; it is broken down. But this divergence in the type of reaction is not universal. With the living bacteria, wherever either locally or generally the infection is resisted or overcome the connective tissue develops more or less steadily until the products of the earlier inflammation are replaced. It then tends to be reabsorbed. If the mass is small it may completely disappear with time; if the mass is large it remains as a scar in no essential particular to be distinguished from a scar due to any other cause.

This reaction of the connective tissue, intimately associated as it is in favorable cases with the healing of tuberculosis, may in the abstract be viewed either as a process dependent in its entirety on other

factors in the disease or it may be considered to be a factor of prime importance in the recovery or partial recovery of the animal. Facts are lacking on which to base the adoption of one or the other of these concepts. For the purposes of this paper, and merely as a working hypothesis, we have adopted the view that the reactive qualities of the connective tissue may be the decisive factor in determining the outcome of a tuberculous infection.

In order to proceed from this point we require some knowledge not at present available of the connective tissue and its reactive possibilities. Is the connective tissue in this sense a constant or a variable for any species of animal? If connective tissue is to be held responsible for variations in reaction to the tubercle bacillus it can only be on the assumption that it has a variable capacity for reaction. Such an assumption is, of course, in accord with the presumption on the broadest biological principles, most, if not all, functions which can be measured being variants in either direction from a typical mean value. In the search for a measure of the capacity of connective tissue to react we have turned to the facts and experiments relating to the healing of wounds.

The repair of any solution of continuity of tissues other than the purely epithelial surfaces involves the production of connective tissue, and attempts have been made to study this reaction quantitatively, particularly, of recent years by Carrel and his associates.[1] A careful consideration of the conditions of these studies makes it seem unlikely that the values determined have any direct relation to the reaction of connective tissue.

As an example we may select an incised wound made through the skin into the soft parts and allowed to gape during the healing process. The gaping wound will fill with exudate which will then become organized with granulation tissue, and this will subsequently be replaced by connective tissue. When the gape or cavity has become well filled with granulations the epithelium will begin to cover the wound by extension from the margins. It is the rate of growth of the epithelium which has been the subject of the quantitative studies above mentioned. Granted that this growth of the epithelium is dependent

[1] Carrel, A., du Noüy, P. L., and Carrel, A., Cicatrization of wounds. IX, *J. Exp. Med.*, 1917, xxvi, 297.

on the state of the underlying connective tissue or granulation tissue as the case may be, it is yet most unlikely that the rate of epithelial growth indicates either the rate of the preceding repair of the subcutaneous tissues or its functional perfection. If conditions could be precisely defined it is probable that in the whole process of wound healing the period when the deep defect was being replaced by potential connective tissue would take its place as a latent period in the reaction of the epithelium.

We have found one account in the literature of an experiment made to measure the effectiveness of connective tissue repair. Sir James Paget[2] (1853) writes as follows:

"The strength, both of the new substance itself, and of its connection by intermingling with the original substance, is worthy of remark. To test it, I removed from a rabbit an Achilles tendon, which had been divided six days previously, and of which the retracted ends were connected by a bond of the size and texture usual at that period of the reparative process. I suspended from the half-section of this bond gradually increased weights. At length it bore a weight of ten pounds, but presently gave way with it: yet we may suppose the whole thickness of the bond would have borne twenty pounds. In another experiment, I tried the strength of a bond of connection which had been ten days forming: this, after bearing suspended weights of twenty, thirty, forty, and fifty pounds, was torn with fifty-six pounds. But surely the strength it showed was very wonderful, when we remember that it was no more than two lines in its chief diameter; and that it was wholly formed and organized in ten days, in the leg of a rabbit scarcely more than a pound in weight. With its tenacity it had acquired much of the inextensibility of the natural tendon. It was indeed stretched by the heavy weights suspended from it, yet so slightly that I think no exertion of which the rabbit was capable would have sufficed to extend it in any appreciable degree."

While these experiments were directed toward a study of the repair of tendons a superficial review of the conditions suffices to show that in the early days following tendon section it must be a value for connective tissue which is determined. This connective tissue may or may not be wholly representative, but it is difficult to devise a better experimental test for the reactive capacity of connective tissue. We therefore adopted the general method of Sir James Paget's experiments as a basis for further work. Our work falls naturally into

[2] Paget, J., Lectures on surgical pathology, London, 1853, i, 271.

two phases: (1) an effort to determine the rate of replacement of the tendon as measured by the strength of the connective tissue laid down between the cut ends; (2) an attempt to use the method in the study of the action of certain chemicals locally or generally applied and for the examination of that phase of the tuberculosis problem previously discussed.

Method.

Adult rabbits were anesthetized with ether. With various degrees of aseptic precaution the Achilles tendon was exposed and sectioned. At various intervals thereafter the animal was killed and the leg amputated at the hip and suspended by the knee through a strong wire loop as an intermediary to a stout chain hung from the ceiling. With a similar wire loop a pail was suspended from the hock. Into this pail clean sand was run from a funnel at a uniform rate and from a uniform height. When a break occurred the flow of sand was stopped, the pail, its contained sand, and all the apparatus coming away with the foot were weighed, the result being recorded in grams as the tensile strength of the newly formed tissue.

At the time the leg was amputated the tendon was dissected free from the surrounding tissue and covered with gauze wet with salt solution. After the leg was hung up, as a last step before the sand was run in, the tibia and the remaining soft parts were cut through with bone forceps and scissors. In this way the new tissue was protected against any irregular strain during the time of preparation.

In general the method can be said to have served its purpose. The difficulties and irregularities for the most part seem to be connected with the surgical procedure. Infection must be avoided as the presence of small pockets of pus in the connective tissue naturally weakens it. Hemorrhage at the time the tendon is cut cannot always be controlled and when the test is made the instances in which evidence of hemorrhage persists must be excluded. The tendon sheath must be carefully sutured at the time of operation. If this is not done the exudate extends through the opening and infiltrates the surrounding fascia. This then organizes and forms adhesions which may involve all the structures from the newly formed matrix of the tendon outward to the skin. On dissection it then is impossible to

isolate the representative tissue. The new connective tissue seems to spring from the old tendon sheath as well as from the cut ends of the tendon, or at least adhesions to the old tendon sheath are universal. If when the dissection is done an attempt is made to remove the last traces of old connective tissue, splits and shreds will be run down into the new tissue which would naturally weaken it. With care, however, the old tissue can be reduced to a thin film without damage to the new. It is not difficult to avoid the gross sources of error, and the variations due to minor faults probably do not affect the results in the main.

Experiments Bearing on the Healing of Wounds.

In the first sets of experiments, those intended to determine the general feasibility of the method, surgical cleanliness was sought through the use of antiseptic solutions. A coal-tar disinfectant of the creolin type was used in solution of appropriate strength for cleansing the skin, keeping the hair wet, and cleansing the hands and instruments. Ordinarily care was taken to touch the field of operation with instruments only and these were freed from excess of antiseptic. Protocols selected as being representative are summarized in Tables I and II.

The experiments recorded in Tables I and II were, as stated above, carried out depending upon an antiseptic solution for surgical cleanliness. In Table III is shown the result of an experiment in which the operative work was done with rigid aseptic precautions. All material used was previously sterilized in the autoclave. Gloves were worn and at the same time especial precautions were taken to keep dust and bits of hair from the skin of the animals from contaminating the wound. In this experiment dichloramine-T is again introduced.

It is not our present purpose to discuss the results of the three experiments presented so far with any pretense to finality.

The results of the second series are in accord with the expectation we entertained when benzene was administered to part of the rabbits. Benzene is known to cause an extensive aplasia of the lymphoid tissue and to give rise to an extreme leucopenia. It was expected to influ-

ence in a deleterious way the exudation processes involved in the repair or replacement of the tendon. The experiment, if its results are accepted at their face value, and we see no reason why they should not be so accepted, indicates that the method is adequate in that it is capable of revealing the action of extraneous influences brought to bear on the reparative process through systemic channels.

It will be noted that there is a large difference between the average tensile strength found in Tables II and III and that that in

TABLE I.

The Achilles tendons of rabbits were cut by open operation under ether anesthesia. The tendon sheath and skin were sutured separately. In the dichloramine-T series 7½ per cent dichloramine-T dissolved in chlorinated eucalyptol, specific gravity 1.2, was put into the tendon sheath and the remainder of the wound at the operation. A small amount remained when the sutures were tied. Breaking strength determined 10 days after section of tendons.

Untreated.	Dichloramine-T.
gm.	*gm.*
4,120	5,470
6,320	7,170*
6,620	7,520
7,750	9,100
7,900	9,370
8,050	10,520
10,070	12,200
10,600	
Average..... 7,678	8,764

* In this case the connective tissue did not break. The tendon pulled out of the muscle and the recorded strength is considerably too low.

Table I is intermediate to these. The low place of Table II is in part to be explained by the fact that when the first of the series were tested the operator made more effort to trim away all traces of the original connective tissue than has seemed practicable. In order that the experiment might not be lost the method was persisted in even though it seemed somewhat faulty. It is probably true also that there was in this series in general more evidence of slight persistent hemorrhage and low grade infection than in the others. The internal varia-

TABLE II.

Operation as in Table I. The rabbits in the benzene series had 5 cc. of 50 per cent benzene in olive oil administered subcutaneously on the day after operation. Breaking strength determined 7 and 10 days after operation.*

Untreated.		Benzene.	
7 days.	10 days.	7 days.	10 days.
gm.	*gm.*	*gm.*	*gm.*
2,270	3,900	1,900	
2,920	4,140	2,380	
3,120	4,400	2,430	
3,450	5,050	2,540	2,240
3,650	5,350	2,650	2,540
3,850	5,700	2,720	3,350
3,950	5,970	2,950	3,390
4,200	6,120	3,420	3,970
4,950	6,200	4,170	5,750
5,620	6,650	4,270	6,200
Average. 3,798	5,348	2,943	3,917

* This whole experiment was carried out by Mr. R. H. Kennicott, then a special student in the Medical School of the University of Pennsylvania.

TABLE III.

Operation aseptic; otherwise as in Table I. In the dichloramine-T series the statements made in Table I about similar series also apply. Breaking strength determined at 10 days.

Untreated.	Dichloramine-T.
gm.	*gm.*
4,920	4,350
6,450	5,350
7,150	6,650
7,700	7,300
8,170	7,600
9,150	7,650
10,000	7,850
11,170	8,800
12,750	10,000
13,750	12,800
Average..... 9,121	7,835

tion in Table II is, however, no greater than the others, if it is as great.

The increased strength attained in 10 days in Table III contrasted with Table I is, we believe, accounted for by the improvement in operative technique—a careful aseptic method having been substituted for a rather crude procedure in which reliance was placed in an antiseptic solution. The fourth experiment (to be presented later), also done aseptically and after more experience, shows a still further increase in the average tensile strength.

In contrasting these experiments emphasis has been laid on the averages. A scrutiny of the tables shows that the averages are surprisingly representative. Where the average is higher it is not due to the presence of exceptionally high figures for individuals.

The dichloramine-T results are not such as to permit satisfactory interpretation. They might be taken to indicate that this material helped in the case of an imperfectly carried out antisepsis and hindered healing when asepsis was better. This substance is, in any event, not one which would be recommended to be left in wounds which are closed tightly as it is capable of causing an aseptic inflammation in the solvent used. Our purpose in including these figures at present is to make available all our data.

As a general method for the quantitative study of a fundamental aspect of wound healing we cannot feel that we have contributed anything in finished form. The wide variation in values found necessitates work on a statistical basis and if there prove to be no other limitation the method is obviously laborious.

Experiment on Tuberculosis.

Having in mind the possible place of the reactivity of the connective tissue in relation to the healing of tuberculosis as presented in the introductory paragraphs of this paper, we sought information by the application of the method as developed to the problem along the following lines. The fact that the length of life of animals of a given species inoculated with anything less than an overwhelming dose of virulent tubercle bacilli varies widely has recently been emphasized by the work of our laboratory. Whatever the reason may be the

strength of the newly formed connective tissue as determined above also varies over a wide range. We sought to determine whether the variations in the two cases were parallel or otherwise. With this purpose in mind the following experiment was carried out.

Young well grown rabbits, weighing about 1,500 gm., were chosen. The Achilles tendon of one leg was sectioned. On the 10th day the leg was amputated at the hip and the tensile strength of the reparative tissue was tested as described. The amputation was carefully conducted under ether anesthesia. Time additional to that needed

TABLE IV.

The rabbits weighed approximately 1,500 gm. The tendons were cut between October 1 and November 15, 1917, under ether anesthesia. Leg amputated and tensile strength of connective tissue determined on the 10th day. March 1, 1918. Inoculated all surviving animals with 0.02 mg. of tuberculosis culture, Bov. C., intravenously.

Length of life after inoculation of tubercle bacilli.	Tensile strength.	Length of life after inoculation of tubercle bacilli.	Tensile strength.
days	*gm.*	*days*	*gm.*
43	8,220	62	10,500
45	6,420	62	8,200
45	12,500	62	6,850
45	9,600	85	7,550
48	12,600	88	14,000
51	11,400	92	13,100
52	11,550	92	14,700
55	8,300	92	8,800
57	9,070	113	11,200
58	13,100	113	9,900
Average strength of first half to die	10,276	Average strength of second half to die	10,480

for healing of the operation wound was allowed for the perfect recovery of the animal from the effects of this precedure. The animals were then inoculated intravenously with a suspension of virulent tubercle bacilli (0.02 mg. of the growth on glycerol agar). Every effort was made to attain uniformity in this inoculation. The animals were then retained until they succumbed to the tuberculosis. In Table IV the length of life of the rabbits in days after the inoculation with tubercle bacilli is set over against the tensile strength of the

connective tissue in grams. Calculation shows that the average tensile strength of the connective tissue formed by the first ten rabbits to die is practically identical with that formed by the last ten animals.

It is apparent that no correlation appears, that consequently we have by this experiment demonstrated no relation between the reactivity of connective tissue in a normal reparative process and the general resistance of the animal to experimental infection with *Bacillus tuberculosis*. In fact the experiment seems sufficiently decisive to warrant the tentative conclusion that no relation exists between any dominant factor in the laying down of connective tissue in response to traumatism and natural resistance to tuberculosis.

SUMMARY.

1. A method has been revived which enables the connective tissue factor in wound healing to be studied quantitatively.

2. It has been found that the functional value of connective tissue formed in response to traumatism as represented by its tensile strength varies widely in different animals.

3. It is suggested that the method may find application in the study of extraneous influences which may affect wound healing either through local or systemic application.

4. It has been determined that there is no parallelism between an active connective tissue response to traumatism and natural resistance to inoculation tuberculosis in the rabbit.

PERSISTENCE OF THE VIRUS OF POLIOMYELITIS IN THE NASOPHARYNX.

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(Received for publication, February 15, 1919.)

This paper is intended as a contribution to the epidemiology of poliomyelitis. Present knowledge places that disease among the infections in which the specific cause is carried in the nasopharynx, and present belief is to the effect that the virus is conveyed from one person to another through the medium of the nasopharyngeal secretions. Indeed, the virus has been detected in these secretions by the inoculation test in three sets of conditions; (1) coincidentally with an attack of the disease incited by it; (2) a considerable period after the attack of the acute disease has abated; and (3) in healthy persons who have been in contact with cases of poliomyelitis.

This determination having been made, detailed information covering the frequency with which and the conditions under which the virus can be discovered in the nasopharynx is greatly to be desired. This information is essential to the working out of the principles of the method of control of epidemic poliomyelitis.

Account must be taken, at the outset, of the difficulties surrounding, at the present time, the demonstration of the virus. The only reliable means of its detection is the inoculation test. When an active virus is injected intracerebrally or even otherwise into monkeys a train of symptoms tends to be set up in these animals which closely resembles the symptoms present in man, the subject of poliomyelitis. Indeed, the analogy is even closer than this, because the histological changes arising in the central nervous organs of the monkeys are an exact counterpart of those present in fatal cases of the disease in human beings. We can now state unreservedly that when typical symptoms appear in the inoculated monkeys typical lesions will occur in their nervous organs.

There is no division of opinion among observers regarding the clinical and histological evidences of poliomyelitis in monkeys in instances in which the experimental disease conforms to frank cases of poliomyelitis occurring in man. There is, however, lack of agreement respecting a less typical experimental condition described by Kling, Pettersson, and Wernstedt.[1] The questions raised by these experimenters, who have introduced entirely new criteria into the subject, are of far reaching significance, because they affect the character of the evidence acceptable as indicating the presence of the virus of poliomyelitis in the nasopharynx. This aspect of the general subject will come under consideration in connection with the experiments to be reported and discussed in the present paper.

Review of the Literature.

In order that the experiments to be described may take their proper place among the studies dealing with the mode of infection in poliomyelitis, a brief review of the literature, especially of that stressing the nasopharynx as the portal of entry and of exit of the virus, will be given.

That the virus, the existence of which at that time was merely suspected, is communicated by personal contact was the thesis which Wickman's[2] studies served to emphasize. But until Landsteiner and Popper[3] communicated the disease to monkeys no more precise definition of the mode of infection could be given. It will be recalled that Landsteiner and Popper originally conveyed the infection from man to monkey by the intraperitoneal route of inoculation and were thus able to reproduce poliomyelitis only in the first series of injected animals. The employment of the intracerebral route of inoculation by Flexner and Lewis[4] led to the discovery that the virus could be transmitted from monkey to monkey through an indefinite series, in course of which its activity or virulence for monkeys increased many fold. Moreover, still other portals of experimental infection were successively disclosed, such as the large nerves, subcutis, subarachnoid space, nasal mucosa, eye, and, although with far greater difficulty, the general blood.

[1] Kling, C., Pettersson, A., and Wernstedt, W., *Communications Inst. méd. État Stockholm*, 1912, iii, 4.

[2] Wickman, I., Beiträge zur Kenntnis der Heine-Medinschen Krankheit, Berlin, 1907.

[3] Landsteiner, K., and Popper, E., *Z. Immunitätsforsch., Orig.*, 1909, ii, 377.

[4] Flexner, S., and Lewis, P. A., *J. Am. Med. Assn.*, 1909, liii, 1639.

In addition, it was found by Leiner and von Wiesner[5] that the virus would penetrate the mucous membrane of the gastrointestinal tract of monkeys in which the motions of the digestive organs were for a time arrested by means of opium. It is noteworthy that aside from the intracerebral route the other modes of infection give more or less inconstant results, and that the external portal which is most favorable to the attack of the virus is the nasal mucous membranes.

The particularly favorable position afforded by the nasal route to infection is a fact the demonstration of which was indicated by circumstances surrounding the epidemiology of poliomyelitis in man. Flexner[6] pointed out that the not infrequent confusion of epidemics of poliomyelitis with those of cerebrospinal meningitis argued for a similarity not only in certain cardinal symptoms but also in the conditions under which the two diseases arise. Thus each disease attacked by preference infants and young children, although not wholly sparing older children and adults. Usually a single case appears in a family or home, but sometimes two cases, and less often three or more appear. These resemblances came to be further emphasized by the detection of the meningococcus carrier on the one hand and the demonstration of the ambulant and abortive cases of poliomyelitis on the other. Indeed, the single striking epidemiological point of difference relates to the seasonal prevalence which for poliomyelitis tends to be summer and autumn and for cerebrospinal meningitis winter and spring. Even this distinction is not absolute; we now know that winter epidemics of poliomyelitis occur, and circumstances favoring epidemics of cerebrospinal meningitis arise in the warm months of the year. The meningococcus carriers have been known for more than a decade; it is important to ascertain whether corresponding poliomyelitic virus carriers exist.

Experience having shown that a highly potent poliomyelitic virus may be secured by successive passages through monkeys, the way was opened for an experimental study of the nasopharynx as the portal of its entry into and exit from the body. The filterability of the virus, moreover, permitted its entire separation from the bacteria present in that locality. Flexner and Lewis,[7] therefore, crushed and extracted in salt solution the excised nasal mucous membrane removed from monkeys which succumbed to intracerebral inoculation of the virus and after filtration through a Berkefeld filter injected the fluid into other *rhesus* monkeys, thus inducing infection and paralysis. The corollary to this experiment, namely the infection of monkeys by direct inoculation of the virus into the nasal mucosa, was quickly supplied by Landsteiner and Levaditi.[8] The last test has been carried out successfully in several ways: by applying the virus to the abraded mucous membrane, by introducing it upon cotton tampons, and

[5] Leiner, C., and von Wiesner, R., *Wien. klin. Woch.*, 1910, xxiii, 91.

[6] Flexner, S., *J. Am. Med. Assn.*, 1910, lv, 1105.

[7] Flexner, S., and Lewis, P. A., *J. Am. Med. Assn.*, 1910, liv, 535.

[8] Landsteiner, K., and Levaditi, C., *Ann. Inst. Pasteur*, 1910, xxiv, 833.

finally by Leiner and von Wiesner[9] by merely pencilling the mucosa without causing any demonstrable lesion whatever. It should be stated, also, that one or the other of the modes of inoculation succeeds or not according, apparently, to the initial potency of the virus and the species of monkey chosen. Finally, some experimental evidence has been adduced to show that the nasal membranes are better suited to convey the virus than the tonsillar tissue. Levaditi and Danulesco[10] made submucous injections into the two regions and induced infection in the one case and not in the other.

The virus of poliomyelitis exists not only in the tissues but also in the secretions of the nasopharynx of monkeys, as shown by Landsteiner, Levaditi, and Danulesco,[11] who inserted cotton plugs into the nares of paralyzed animals and found that after several hours the absorbed fluid sufficed, when injected, to infect other monkeys; while Thomsen[12] ascertained also that merely rubbing the mucosa with the tampon was sufficient to incite infection. In this connection mention may be made of the fact that Landsteiner and Levaditi[8] consider that they have traced the passage of the virus along the olfactory nerves to the olfactory lobe of the brain, and Flexner and Clark[13] likewise have found the olfactory lobes infective, and the spinal cord and medulla non-infective, 48 hours after an intranasal inoculation of the virus. The last observation indicates that the penetration of the virus from the surface to the interior of the nasal membrane and thence to the brain occurs quickly, a point borne out by certain experiments made with antiseptic drugs to be reported elsewhere.

The foregoing experimental results provide a basis for considering the inoculations which have been made directly with human materials derived from the nasopharynx. These materials may be divided into three classes as follows: (1) washings, (2) tissues (tonsils and adenoids) removed during life, and (3) tissues (tonsils, pharyngeal mucosa, nasal mucosa) recovered after death. As regards the first, numerous tests have been made. It will be of doubtful value to note in this place the failure to induce infection. The negative results are, in the light of present knowledge, without real significance. The limits of activity for monkeys of virus immediately obtained from human beings are quickly reached. Hence the dilute filtered washings of the nasopharynx could be expected to succeed only rarely. We have ourselves failed to incite infection with a filtrate prepared from the spinal cord of human cases of poliomyelitis when the unfiltered suspensions were active.

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[9] Leiner, C., and von Wiesner, R., *Wien. klin. Woch.*, 1910, xxiii, 323.

[10] Levaditi, C., and Danulesco, V., *Compt. rend. Soc. biol.*, 1912, lxxii, 606.

[11] Landsteiner, Levaditi, and Danulesco, *Compt. rend. Soc. biol.*, 1911, lxxi, 558.

[12] Thomsen, O., *Berl. klin. Woch.*, 1912, xlix, 63.

[13] Flexner, S., and Clark, P. F., *Proc. Soc. Exp. Biol. and Med.*, 1912–13, x, 1.

However, Kling, Pettersson, and Wernstedt[1] have brought indubitable proof that washings obtained from the nasopharynx both during life from typical cases of poliomyelitis and after death from persons who succumbed to the disease, may carry the virus in a form and quantity capable of inciting infection in monkeys. If we confine ourselves strictly to the instances in which the washings conveyed typical experimental poliomyelitis, it may be said that while the number of failures to infect exceeds the successful inoculations, yet the latter comprise a convincing series. While opinion on this point can hardly be divided, the same cannot be stated for the interpretation which they place upon the results of the inoculation of washings from abortive cases, mere contacts, and from recovered persons, to which fuller reference will be made presently.

The first to detect the virus in tonsillar and pharyngeal mucosa obtained from a fatal case of poliomyelitis in man and thus to confirm Flexner and Lewis' experiments on the monkey were Landsteiner, Levaditi, and Pastia.[14] Flexner and Clark[15] about the same time reported several similar successful inoculations. The latter authors drew attention to the fact that when they injected filtrates of the tonsillar and nasal tissues infection failed; but when the same materials were rendered bacteria-free with 0.5 per cent phenol and inoculated as suspensions infection followed.

Thus far all the successful inoculations of the virus of poliomyelitis noted upon monkeys have been secured with materials obtained from recent typical cases of the disease in man or the experimental reproduction of that disease in those animals. An experimental answer to the important question of the period of the survival of the virus in the nasal and buccal mucosa was first successfully attempted by Lucas and Osgood,[16] who were still able to determine its presence in the experimentally infected monkey at the expiration of nearly 6 months.

Wickman's important studies[2] pointed unmistakably to the part played by so called abortive cases of poliomyelitis and healthy carriers of the microbic cause in disseminating poliomyelitis. The discovery of the experimental disease in monkeys led to the search for the virus in the nasopharynx of those two classes of persons. By far the most extensive inoculation tests published are those of Kling, Pettersson, and Wernstedt.[1] They even go beyond the phases of the subject indicated and include an investigation of the period of survival of the virus in the nasopharynx. Unfortunately this highly important study is marred by the fact that the criteria of the experimental disease in monkeys which the authors came to adopt do not conform to those which all experimenters accept as distinctive of poliomyelitis in the monkey. The point is an essential one. A close review of their protocols has led us to doubt the validity of their conclusions.

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[14] Landsteiner, K., Levaditi, C., and Pastia, C., *Semaine méd.*, 1911, xxxi, 296.
[15] Flexner, S., and Clark, P. F., *J. Am. Med. Assn.*, 1911, lvii, 1685.
[16] Lucas, W. P., and Osgood, R. B., *J. Am. Med. Assn.*, 1913, lx, 1611.

The inoculation experiments which form the basis of this paper deal with this aspect of the subject.

Meanwhile, it has been shown through inoculation in one instance by Flexner, Clark, and Fraser[17] that healthy persons may harbor the virus in their nasal and buccal secretions. The instance in which the demonstration was made related to the parents of a young child acutely ill with poliomyelitis. The contact, therefore, between the carriers and the case was an intimate one. This unquestioned observation was followed by a similarly conclusive one by Kling and Pettersson,[18] who, it may be remarked in passing, attribute failure to incite typical clinical and anatomical effects in their earlier experiments to the injections of insufficient amounts of the virus. Finally, Taylor and Amoss[19] have recorded two instances—one an abortive or non-paralytic case of poliomyelitis and the other a healthy child who subsequently developed frank paralysis—in which the concentrated nasopharyngeal washings inoculated into monkeys induced the typical experimental disease.

Nature of Materials and Interpretation of Experiments.

The experiments which we are reporting were made with human materials of two sorts; namely, tonsils and adenoids removed during life and tonsils and pharyngeal mucosa excised after death. The object in all the experiments was to ascertain the presence or absence of the virus as far as this could be determined by the inoculation test. In this way it was hoped to throw light on the persistence of the virus in the nasal and buccal membranes and their discharges.

The outcome of the tests is not so consistent as we would wish. But the inconsistency which will appear is instructive, for not only does it point the limitations of the inoculation test, but it helps clear up, at the same time, the more indefinite clinical effects which sometimes follow the injections of supposedly virus-containing materials. It may be mentioned here that we did not confirm the experiments of Kling, Pettersson, and Wernstedt respecting an atypical variety of poliomyelitis in the monkey which they finally came to attribute to the inoculation of too little virus-containing washings. Our tests, as will appear, were carried out with tissues and with quantities that should have sufficed for infection had the virus been present in amounts to be expected and in an active state.

[17] Flexner, S., Clark, P. F., and Fraser, F. R., *J. Am. Med. Assn.*, 1913, lx, 201.
[18] Kling, C., and Pettersson, A., *Deutsch. med. Woch.*, 1914, xl, 320.
[19] Taylor, E., and Amoss, H. L., *J. Exp. Med.*, 1917, xxvi, 745.

The last considerations are pertinent because of the striking contrast in the results obtained with human tissues removed either by operation or post mortem. The obvious deduction is to the effect that the upper respiratory mucosa carries far more active virus in the latter instance. All the postmortem tissues inoculated by us were derived from acute cases. Analogy with other acute infections leads to the assumption that in the fatal instances there is more active multiplication of the virus than in cases which go on to recovery. This is perhaps the simplest interpretation to put upon our tests.

The discrepancy seems to illuminate the question as to the time of maximum infectivity in epidemic poliomyelitis. As far as observations made during the great epidemic, which prevailed in New York state and elsewhere in 1916, can be construed, the implication is to the effect that this period is relatively brief and is greatest early in the disease. It cannot be said, however, that this point is definitely established. As a matter of fact, the literature contains records which directly controvert this idea. The matter is perhaps one not to be settled absolutely but rather to be placed on a relative basis. What we need first to know are the conditions under which the virus of poliomyelitis can be definitely determined to be present in the nasopharynx. This question we have endeavored to answer by a study of tissues removed post mortem and of hypertrophied tonsils and adenoids extirpated during life from persons who had suffered a typical attack of poliomyelitis some weeks or months earlier. In comparing the two classes of tissue, attention must be given the fact that the former, that is the materials removed post mortem, come from cases earlier in the course of the disease.

The results of the inoculation of the tonsillar and adenoid tissues removed during life have also an important bearing on the question of the chronic carriage of the virus upon the nasopharynx. The test of Lucas and Osgood[16] showed that the virus may be demonstrable on the nasopharyngeal mucous membrane of the monkey as long as 5 months after infection had been experimentally induced. Flexner and Clark[20] demonstrated it in the nasopharyngeal mucosa of a monkey surviving the paralysis more than 4 weeks and failed, in the

[20] Flexner, S., and Clark, P. F., *J. Am. Med. Assn.*, 1911, lvi, 585.

same animal, to detect it in the spinal cord. The large series of tests with washings from human cases made by Kling, Pettersson, and Wernstedt[1] on the basis of which they believe they have shown chronic carriage of the virus to be common, have never been subjected to a searching control with human tissues. Our experiments with surgically removed tonsils and adenoids may be regarded as covering this point. It happens, indeed, that our results are quite opposed to those of the Swedish observers and teach rather that the chronic carriage is, at least, exceptional. On the whole the epidemiological data are in conformity with our experimental results.

The Swedish authors studied nine convalescents over a maximum period of 7 months on the basis of which they formulated the following deductions: "that the secretion from the mucous membranes of the mouth and intestine of persons who have recovered (from poliomyelitis) has had the power of infecting monkeys still several months, in one case 204 days (nearly 7 months), after the onset of the illness, giving rise to an experimental poliomyelitis with fatal issue . . . . It was only in one case that we did not succeed in demonstrating the presence of the virus after the comparatively short time of 30 days."[21]

In interpreting this statement and in considering the discrepancy with our studies, it is necessary to take into account the immediately succeeding paragraphs:

"During the time occupied by the investigations, the virus had changed its character, so that it no longer caused inflammations with cellular exudations. Instead of this the degeneration of the nerve cells, the changes of the glia cells and the neurophagocytosis caused by the enlarged glia cells have been the characteristic changes. They have thus been of the same type as those appearing in the monkeys injected with secretions from abortive cases, and virus carriers, changes which . . . . we consider ourselves justified in assuming to be due to a less virulent virus. *The experiment also shows, that the microbe rather quickly—already after 8–14 days—loses its power of causing inflammatory exudations in the inoculated animals. This fact is of very great importance from a practical point of view since it perhaps gives us the right to assume that the virus, possibly rather soon after the termination of the acute stage, gets weaker.*"[22] (Author's italics.)

We propose now to present our results in the form of tabulations with such discussion as seems called for. The postmortem tissues

[21] Kling, Pettersson, and Wernstedt,[1] p. 159.

[22] Kling, Pettersson, and Wernstedt,[1] pp. 159–160.

employed for inoculation were obtained very soon after the death of the patient, when they were either inoculated after a short interval, or placed in the preserving fluids, which consisted of 50 per cent sterile glycerol or 0.5 per cent phenol. The tissues removed surgically were put immediately into the preservative fluids. The inoculations were made at leisure. The glycerolated or phenolized specimens were suspended and injected partly intracerebrally and partly intraperitoneally. Previous experiments had shown that the weak phenol destroyed associated bacteria in the tissues without acting appreciably upon the virus of poliomyelitis.[15] In this respect the phenol was superior to the glycerol, which also was without observed injurious action on the virus, but which removed the bacteria much more slowly.

Histological studies were made with the nervous organs of all monkeys succumbing to or after the inoculations. All the animals which developed typically clinical poliomyelitis showed characteristic histological lesions in the spinal cord and medulla and intervertebral ganglia. In none of the animals in which the clinical symptoms were dubious did we find histological lesions either resembling those of typical poliomyelitis or corresponding with the degenerative and peculiar neurophagocytic ones which Kling, Pettersson, and Wernstedt[1] describe and attribute to the action of specifically weakened poliomyelitic virus.

Examination of Table I brings out the fact that both the tonsils and nasal mucosa of fatal early cases of poliomyelitis are infectious for monkeys. No real distinction can be drawn between the two sets of materials as regards their infectivity. The fact that now one tissue from an individual succeeds while the other fails is probably as ascribable to differences in the susceptibility of the individual monkeys as to irregularity in distribution of the virus. The inferiority of filtrates to emulsions or suspensions of the tissues is manifest. It is clear also that phenol is a favorable medium for preparation of the tissues for inoculation, as it tends quickly to destroy bacteria associated with the virus without materially injuring the virus itself. Probably the 0.5 per cent phenol is only relatively innocuous for the virus, since in the instance of H. K., in which two *rhesus* monkeys were inoculated,

TABLE I.

Human Tissues Obtained post Mortem.

Case.	Day of disease.	Monkeys inoculated.	Material injected.	Symptoms.	Histology.	Remarks.
J. A. R.	6	2 *M. rhesus.*	Berkefeld filtrate of tonsils and pharynx mucosa.	None.		Filtrate of spinal cord negative; emulsion positive.
R. P.	7	2 *M.* "	(1) Emulsion of tonsils. (2) Emulsion of nasal mucosa.	Tonsils none. Nasal mucosa typical.	Typical lesions from nasal mucosa.	Glycerol preservation. Nasal mucosa positive. Tonsils negative.
M. K.	6	2 *M.* "	Heim filtrate of tonsils and nasal mucosa separately.	None.		Glycerol preservation.
J. C.	3	1 *M.* " 1 *M. cynomolgus.*	Heim filtrate of tonsils and nasal mucosa separately.	Nasal mucosa none. Tonsils typical.	Typical lesions from tonsils.	Glycerol preservation. Tonsils positive in *M. cynomolgus.* Nasal mucosa negative.
B. T.	6	1 *M. rhesus.*	Emulsion of tonsils.	Typical.	Typical lesions.	Phenol preservation. Reinoculation of spinal cord positive.
J. G.	7	1 *M.* "	" " "	"	" "	Phenol preservation.
S. A.	9	2 *M.* "	" " " and pharynx mucosa separately.	None.		" "

H. K.	6	2 *M. rhesus*	Emulsion of tonsils.	Typical, with recovery.		Phenol preservation in successive quantities to remove resistant bacteria.
G. G.	6	3 *M.* "	" " "	Typical.	Typical lesions.	Phenol preservation.
A. K.	8	1 *M.* "	" " "	None.		
J. W.	4	1 *M.* "	" " " and pharynx mucosa.	"		
G. C.	?	1 *M.* "	Heim filtrate of tonsils.	Indefinite.	Tuberculosis.	
B. H.	10	1 *M.* "	Heim filtrate of tonsils.	None.		
A. S.	3	1 *M.* "	Emulsion of tonsils intrasciatic and intraperitoneal.	"		Glycerol preservation.

TABLE II.

Human Tissues Removed Surgically.

Case.	Day of disease.	Monkeys inoculated.	Material injected.	Symptoms.	Histology.	Remarks.
H. G.	8	1 *M. rhesus.*	Berkefeld filtrate of tonsils and adenoids.	None.		
I. P.	13	1 *M.* "	Emulsion of tonsils and adenoids.	Gradual emaciation with diarrhea. Died 15th day.	No lesions in nervous organs.	Phenol preservation.
A. S.	13	1 *M.* "	Heim filtrate of tonsils and adenoids.	Indefinite. Recovered.		
J. P.	13	1 *M.* "	Emulsion of tonsils and adenoids.	None.		Glycerol and phenol preservation.
H. B.	14	1 *M.* "	Emulsion of tonsils and adenoids.	Emaciation. Died 15th day.	Tuberculosis.	Phenol preservation.
C. M.	17	1 *M.* "	Filtrate of tonsils and adenoids.	None.		" "
H. N.	18	2 *M.* "	Emulsion of tonsils and adenoids.	"		
C. U.	19	2 *M.* "	Berkefeld filtrate of tonsils and adenoids.	"		
J. A.	19	2 *M.* "	Emulsion of tonsils and adenoids.	Indefinite.	Tubercles in spinal cord.	Glycerol preservation. Succumbed to tuberculosis.
F. S.	22	1 *M.* "	Berkefeld filtrate of tonsils and adenoids.	None.		

J. W.	25	2 *M. rhesus.*	Emulsion of tonsils and adenoids.	None.		Glycerol preservation.
H. F.	25	2 *M.* "	Emulsion of tonsils and adenoids.	(1) None. (2) Pressure. Symptoms from which recovered. Then indefinite. Death on 12th day.	No lesions suggestive of poliomyelitis. No degeneration of nerve cells in medulla, cord, or intervertebral ganglia, and no cellular infiltration.	Glycerol preservation. Small sterile cyst at point of inoculation.
F. S.	27	2 *M.* "	Emulsion of tonsils and adenoids.	None.		Glycerol preservation.
L. F.	27	2 *M.* "	Emulsion of tonsils and adenoids.	"		Glycerol preservation.
A. L.	27	1 *M. cynomolgus.*	Emulsion of tonsils and adenoids.	"		Phenol preservation.
C. J.	28	1 *M. rhesus.*	Filtrate of tonsils and adenoids.	"		
H. F. S.	28	2 *M.* "	Berkefeld filtrate of tonsils and adenoids.	"		
A. S.	35	1 *M.* "	Berkefeld filtrate of tonsils.	"		
G. C.	35	1 *M.* "	Emulsion of tonsils and adenoids.	"		Phenol preservation.
T. P.	39	1 *M.* "	Filtrate of tonsils and adenoids.	Indefinite. Recovered.		" "

TABLE II—*Concluded.*

Case.	Day of disease.	Monkeys inoculated.	Material injected.	Symptoms.	Histology.	Remarks.
J. O'B.	49	2 *M. rhesus.*	Emulsion of tonsils and adenoids.	(1) Died suddenly 8th day. (2) Gradually lost strength. Died 15th day.	(1) No lesions in nervous organs. (2) No lesions in nervous organs.	Glycerol preservation.
D. M.	50	2 *M.* "	Emulsion of tonsils and adenoids.	(1) None. (2) Indefinite.	(2) No lesions in nervous organs.	Glycerol preservation 5 days. Cyst in cerebrum at point of inoculation.
F. McK.	50	2 *M.* "	Emulsion of tonsils and adenoids.	(1) Typical. Died 41st day. (2) None.	(1) No lesions in nervous organs.	Phenol preservation.
T. M.	90	1 *M.* "	Emulsion of tonsils and adenoids.	None.		Phenol preservation. Reinoculation with emulsion.
J. W.	90	1 *M.* "	Emulsion of tonsils and adenoids.	Died suddenly $2\frac{1}{2}$ mos. after 1st and 19 days after 2nd inoculation.	No lesions in central nervous organs.	Phenol preservation. Reinoculation with emulsion.
M. K.	120	1 *M.* "	Emulsion of tonsils and adenoids.	Indefinite after 2nd inoculation. Recovered.		Phenol preservation. Reinoculation with emulsion.
E. S.	150	1 *M.* "	Emulsion of tonsils and adenoids.	Indefinite after 2nd inoculation. Recovered.		Phenol preservation. Reinoculation with emulsion.

and in which the tissues employed were put through three successive solutions of the phenol to render them approximately bacteria-free, a milder form of poliomyelitis developed. The number of tests made is insufficient to account for certain obvious discrepancies as, for example, the failure of tissues derived from cases dying on the 3rd and 4th day of the disease to infect. Doubtless the fact that only one monkey was used in each instance for inoculation had something to do with the failures. But at times the supply of monkeys was too small and precarious to permit of more being tested. Experience with inoculations of the spinal cord and medulla from human cases has shown us that the failure to incite infection in a single *rhesus* monkey does not indicate lack of power to infect still other individual animals of the same species.

The deduction from the tests summarized in the table is to the effect that the nasal and pharyngeal mucosæ of persons succumbing to poliomyelitis during the 1st week or 10 days of the disease probably regularly contain the poliomyelitic virus.

A glance at Table II shows at once a fundamental difference in the results of inoculating the specimens removed surgically and those obtained at autopsy. The extent of the distinction is not greatly lessened by the probability that the tests carried out with the surgical tissues err on the side of negativity. That certain of the surgical specimens contained virus in some amount we think most probable. But the essential fact remains that under the conditions of the experiments they regularly failed to incite infection and paralysis in the monkeys.

The two sets of tissues, those removed post mortem and those removed during life, differed especially in one respect; namely, that the former came from cases of poliomyelitis in the 1st week and the latter later in the course of the disease. We are inclined to attribute to this circumstance, with which may be associated the tendency for microorganisms to multiply more freely in the last hours of life, the great differences in effects observed.

In order to favor the induction of infection with the specimens removed relatively late after recovery from the acute symptoms of poliomyelitis, the reinoculation method of Flexner, Noguchi, and

Amoss[23] was also employed. By this method it is possible to convert a subminimal infective dose of the virus into an effective dose. But still no success was achieved with the surgical tissues.

DISCUSSION.

The epidemiology of poliomyelitis has still to be worked out in detail, as many factors governing the spread of the disease remain to be discovered. That the virus or microbic cause is communicated by personal contact is now generally admitted. That the virus occurs in the nasopharynx, which constitutes the chief locus of ingress and egress to and from the body is also conceded. The fact that the virus has been, if rarely, detected in healthy persons who have been in intimate contact with early cases of poliomyelitis, and even in certain individuals who have recovered from the acute effects of the disease, has led to the generalization that like some other diseases of bacterial origin, and notably epidemic meningitis, healthy and chronic carriers of the virus are frequent. This view has received its main support from Kling, Pettersson, and Wernstedt, whose studies we have discussed. A critical analysis of the basis of their contention fails, however, to carry conviction, and the doubt which has arisen as to the true interpretation of their results is deepened, we think, by our more searching tests.

The results of the experiments reported in this paper conform closely with clinical experience in the United States, at least, and especially with the observations made by epidemiologists in the course of the wide epidemic in New York State and elsewhere during the summer and autumn of 1916. The conclusion reached at that time was to the effect that the communicability of the disease was a phenomenon chiefly of the early stages, while the frankly paralyzed person and the convalescent were to be feared much less. In our experiments infection was secured with tissues obtained during the 1st week, approximately, of the disease but not at the later periods.

CONCLUSIONS.

The virus of poliomyelitis occurs in the nasopharynx of man and monkeys.

[23] Flexner, S., Noguchi, H., and Amoss, H. L., *J. Exp. Med.*, 1915, xxi, 91.

In man it has been detected by the inoculation test in washings from acute cases, rarely in similar washings from healthy contacts, in the nasopharyngeal tissues obtained from fatal cases in the 1st week of infection, but rarely, if ever, from nasopharyngeal tissues removed surgically at later periods in the course of the disease.

In monkeys, also, the virus has been detected in the secretions from acute experimental infections, in the nasopharyngeal tissues derived from early cases, and rarely from cases several weeks or months after recovery from the acute symptoms.

The inoculation of tonsils and adenoids obtained from cases of undoubted poliomyelitis either yielded definite results in the form of typical paralysis and histological lesions in the central nervous organs of the monkeys injected, or no symptoms or lesions which could be confounded with poliomyelitis. The indefinite symptoms and atypical lesions described in a certain class of inoculated animals by Kling, Pettersson, and Wernstedt were not encountered in our experiments.

The deduction from the experiments reported is to the effect that the virus is regularly present in the nasopharynx in cases of poliomyelitis in the first days of illness, and especially in fatal cases; that it diminishes relatively quickly as the disease progresses, except in rare instances; and that it is unusual for a carrier state to be developed. Hence the period of greatest infectivity of patients would appear to be early in the disease, which is probably the time at which communication of the virus from person to person takes place.

Available evidence proves that healthy carriers of the virus occur. We do not, however, possess data which indicate the frequency with which carriage arises. The fact that even after a severe and wide epidemic, such as occurred in the United States in 1916, the disease may virtually disappear within 2 or 3 years, points to the probability that enduring carriers of the active virus, whether healthy or chronic, are of exceptional occurrence.

We are greatly indebted to Dr. Cornelius G. Coakley for many of the specimens of surgically removed tonsils and adenoids with which our experiments were performed. We desire to express sincere appreciation for his cordial cooperation.

I. RENAL FUNCTION INFLUENCED BY INTESTINAL OBSTRUCTION.

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The literature on the intoxication of intestinal obstruction reveals the fact that no direct study has been made of the renal function in this condition. Yet, there is indirect evidence of functional impairment in spite of the fact that the present histological methods fail to show definite alterations in the kidney parenchyma.

Hartwell, Hoguet, and Beekman (1, 2) report finding degeneration of the tubular epithelium at autopsy in many of their dogs dying from experimental obstruction of the intestine, but we assume that these changes were post mortem or were due to some secondary factor or to antecedent disease, for no demonstrable kidney lesions have been found in animals autopsied immediately after death from uncomplicated obstruction in this laboratory.

Indirect evidence of functional derangement is found, however, in the rapid increase in the non-protein nitrogen of the blood during the intoxication, as first reported by Tileston and Comfort (3) in a small number of human cases of obstruction and observed experimentally in dogs with isolated loops of the small intestine or with simple obstruction, by Cooke, Rodenbaugh, and Whipple (4). These observers have shown that the non-coagulable nitrogen of the blood rises rapidly to a high level, often from 100 to 300 per cent above the normal, which level is sustained until death in fatal cases, or until the clinical symptoms of intoxication have subsided in those animals that recover. The creatinine nitrogen shares decidedly in this rise as shown by these same experiments (4), and in this connection we wish to refer to the conclusion of Myers and Lough (5) that: "The creatinin rises above 2.5 mg. per 100 cc. of blood almost without exception only in conditions with renal involvement." In their studies these observers were dealing with cases of true nephritis in which the creatinine of the blood had perhaps accumulated over a relatively long period of time because of a failure on the part of the kidneys to eliminate it at the normal rate. In the case of acute intestinal obstruction, on the other hand, a much more rapid rise in the various non-protein nitrogenous constituents of the blood is made possible by the greatly increased rate of tissue protein catabolism. Upon removing the source of the intoxication it is found that the blood non-protein nitrogen soon

returns to normal again. In spite of this difference, however, between the case of true nephritis and what has been observed in acute intestinal obstruction, the possibility remains that the high values found for the creatinine of the blood in the latter condition may be due in great part to its retention by temporarily disabled kidneys.

At the time that observations (4) were made showing the high blood non-protein nitrogen in intestinal obstruction, it was observed that the level of urinary nitrogen excretion was very high, at times twice or three times normal. This great increase in nitrogen elimination indicated that a large excess of body protein was being broken down and it was thought that this phenomenon might explain the high non-protein nitrogen of the blood. However, it was shown in later experiments (6) that this increased protein breakdown gave the usual products of the normal protein catabolism, observed, for example, after protein feeding. It could not be denied that there was a possibility that small amounts of toxic split products might be formed by this abnormal protein catabolism, but present methods of analysis give no positive evidence for this. In view of the fact that there was no positive proof that toxic substances were formed from the excess protein catabolism, it became very difficult to give any satisfactory explanation for the heaping up of non-protein nitrogen in the blood. It was recalled that the normal kidneys can excrete enormous amounts of normal nitrogenous end-products and even this great protein catabolism of obstruction should be easily taken care of by a pair of normally functioning kidneys.

The above brief statements, therefore, indicate a lack of definite information regarding the eliminative function of the kidneys during periods of intoxication due to intestinal obstruction. It was with the purpose of supplying this information that the present study was undertaken.

Methods.

Dogs, mostly females, were used in all the experiments. Only healthy young adult animals were selected after being under careful observation for several days at least, during which time the urine was examined with reference to its specific gravity, reaction, and albumin and cast content.

The dogs chosen were then allowed in most instances to fast several days (3 to 5) after which various renal function tests were performed on them in exactly the same manner and under the same general conditions which were to obtain in the later experiments. The data recorded at this time for each dog afford a control for the

later experiments on the same animal. Following this preliminary examination of the animal, a simple obstruction of the small intestine was made by section of the intestine with inversion of the cut ends. In Dog 19–55 (Tables VIII and IX) an isolated loop of the ileum was made in the usual way and the remaining ends of the intestine were united by a lateral anastomosis.

Methods for the measurement of renal efficiency were chosen which are simple to perform but which give a reasonably accurate idea of the activity of the kidneys at the moment of examination. None of the longer tests involving special diets or the administration of various substances by mouth could be employed because of the tendency to vomiting and diarrhea during the intoxication.

Since there exists a general concensus of opinion among those who have made a comparative study of the various renal function tests (7, 8, 9) that no single test so far devised is entirely adequate to detect every type of kidney impairment and to distinguish renal injury from the extrarenal factors often involved, we have selected the three following methods: (1) that of measuring the urea-excreting capacity of the kidneys; (2) the phenolsulfonephthalein elimination method of Rowntree and Geraghty (10, 11); (3) that of determining the rate of excretion of injected sodium chloride.

The first method, that of measuring the power of the kidneys to excrete urea, was carried out by determining the blood urea and simultaneously the rate of urea excretion in most instances after the injection of urea. These values have been expressed in the form of the ratio

$$\frac{\text{Urea in 1 hr.'s urine}}{\text{Urea in 100 cc. of blood}}$$

after the method of Addis and Watanabe (12) and Watanabe, Oliver, and Addis (13). These workers propose the use of this simple ratio after the administration of urea for determining the quantity of functioning renal tissue. Under the strain imposed by the administered urea, the kidney with a diminished amount of secreting tissue reveals its lack of reserve power by a lowering of the ratio. They found that the ratio is not lowered by the removal of one kidney in a

normal animal unless urea is given, when it becomes apparent that the maximum capacity to excrete urea is exceeded more readily after the secreting tissue has been cut down one-half.

In the present study there is no process comparable with that of removing a part of the cells and leaving the intact cells in a perfectly normal condition as in the experiments of Addis and Watanabe. Nevertheless, if a large number of the cells are injured so that their individual ability to function is impaired, the effect upon the ratio should be the same, and, if this impairment is very marked, the ratio should fall even when no urea is injected. The urea of both the blood and the urine was determined in our experiments by the Van Slyke-Cullen modification of Marshal's urease method (14, 15).

The phenolsulfonephthalein test was applied according to the procedure outlined by its authors, Rowntree and Geraghty (11). Often during the period of intoxication isotonic saline or glucose solution was injected intravenously and in every case the animal received more fluid during this time than during the corresponding control period, in order that the volume of fluid lost to the body by way of the alimentary tract might be made up in part at least.

In the third method, that of measuring the efficiency of the kidneys in excreting sodium chloride, the salt was injected intravenously in hypertonic solution and its excretion over short periods was followed, as in the case of the injected urea, both before and during the period of intoxication. The total chlorides of the urine were determined by the McLean-Van Slyke method (16).

During the periods of observation the dog was placed in a large metabolism cage fitted with a simple harness which holds the animal during catheterization in a comfortable, fixed position, thereby making this process easy to perform by one person in a few minutes time. The bladder was thoroughly rinsed with lukewarm distilled water just before and at the end of each period. Collection of samples was always begun a few minutes before the end of the period in order that it could be discontinued sharply at the expiration of the time allotted. In cases of diuresis following the injection of urea or saline solution the bladder was catheterized more frequently so that the samples could be collected directly. Night samples alone in the case of the chloride tests were collected in metabolism cages.

EXPERIMENTAL OBSERVATIONS.

Not all the experimental data are submitted because some of the experiments were incomplete or complicated by factors not intended to be present which introduced difficulties in the interpretation of results. We may say, however, that the other experiments which are not tabulated confirm the points made in this communication.

TABLE I.

Dog 18–138. Simple Obstruction of the Ileum. Blood Urea. Phenolsulfonephthalein Elimination.

Date.	Weight.	Blood urea per 100 cc.	Phthalein elimination in 2 hrs.	Remarks.
1918	*lbs.*	*mg.*	*per cent*	
June 18	40.5	31	71	No food given since June 15. Fasting continued.
June 19	Simple obstruction of ileum.			
June 20	38.7		69	Recovery satisfactory.
" 21	37.5	50	49	Dull. Acts sick. Some feces.
" 22	37.0	74	37	Very dull. Intoxication develops rapidly. Vomiting and diarrhea during day. Dead next morning.

TABLE II.

Dog 18–138. Simple Obstruction of the Ileum.

$$\textit{Ratio: } \frac{\textit{Urea in 1 Hr.'s Urine}}{\textit{Urea in 100 Cc. of Blood}}.$$

Date.	Hour.	Urea injected.	Urea excreted per hr.	Blood urea per 100 cc.	Ratio.	Remarks.
1918		*gm.*	*gm.*	*mg.*		
June 18, p.m.	1	20	1.89	123	15.3	Control.
	2		1.61	106	15.1	Marked diuresis.
	3		0.92	91	11.6	
June 19	Simple obstruction of ileum.					
June 22, p.m.	1	20	0.64	212	3.0	Intoxication of intense grade.
	2		0.40	164	2.4	Urine not much increased by injection of urea.
	3		0.35	146	2.4	

Dog 18–138 (*Tables I and II*).—Long haired mongrel, female; weight 40.5 pounds.

June 18, 1918. After 3 days fast 20 gm. of urea dissolved in 118 cc. of distilled water were injected intravenously. Phenolsulfonephthalein was given intramuscularly at the same time. The simple urea ratio

$$\frac{\text{Urea in 1 hr.'s urine}}{\text{Urea in 100 cc. of blood}}$$

was then determined for each of the following 3 hours (Table II).

June 19. A simple obstruction was made 10 cm. above the ileocecal valve.

June 20. Dog was quiet but did not act sick. Small semisolid stool. Temperature 39.5°C.

June 21. Somewhat dull. Appears sick.

June 22. Moderate intoxication in forenoon. More marked in late afternoon. Pulse weak. Vomiting bile-stained mucus. Slight diarrhea late in day. Temperature 38.9°C. Died during the night.

Autopsy.—Lungs and heart normal. Peritoneum clean and glistening. Small intestine distended with gas and a thin, slimy, slate-colored fluid which had a putrid odor. Portions of the mucosa were engorged and velvety. A few petechial hemorrhages were seen in it. Stomach contained slight amount of bile-stained mucus. Liver and spleen moderately engorged with unclotted blood. Kidneys normal in appearance both grossly and microscopically. Remaining viscera likewise negative.

The protocol of Dog 18–138 together with Table I shows the typical acute reaction following a simple uncomplicated obstruction of the small intestine, although death does not ordinarily occur in such a short time. The animal usually recovers from the immediate effects of the operation and may remain active and bright for several days and in certain cases many days. Then rather abruptly, the symptoms of intoxication, namely dullness, temperature reaction, weakness of the pulse, vomiting, and prostration, appear. The dog may remain in this condition for several days before death or may show a rather precipitous decline and die during the night following the first appearance of these symptoms. In either case the blood urea may soar to twice or three times its normal value just before death, rising gradually from day to day in the cases in which the symptoms appear early and very rapidly in the instances of sudden intoxication and death.

Table I shows a gradual increase in the blood urea from day to day

and a corresponding gradual diminution in the percentage of phenolsulfonephthalein eliminated per 2 hours.

In Table II are presented data regarding the effect of the acute intoxication upon the urea-excreting power of the kidneys as measured by the urea ratio. It will be seen from these figures that the excretory function as far as urea is concerned is far below normal. The ratio of the urea excreted per hour to the urea per 100 cc. of blood was lowered from an average normal value of 14 before the obstruction was made to an average value of 2.6 during the height of the intoxication on the day preceding the death of the animal. The decrease in this ratio is, therefore, much more striking than that in the percentage of dye eliminated.

TABLE III.

Dog 19–17. Simple Obstruction of the Ileum. Blood Urea. Phenolsulfonephthalein Elimination.

Date.	Weight.	Blood urea per 100 cc.	Phthalein elimination in 2 hrs.	Remarks.
1918	*lbs.*	*mg.*	*per cent*	
Aug. 8	30.3	28	70	Dog fasted 3 previous days.
" 9	30.2	27	72	
Aug. 10	Simple obstruction just above ileocecal valve.			
Aug. 11	29.0	24	67	Light attack of distemper. Recovery from operation satisfactory.
" 12	28.5	41	63	Distemper worse. Intoxication definite in p.m.
" 13	27.3	97	Mere trace.	220 cc. of $\frac{M}{6}$ salt solution given intravenously soon after dye injection. Vomits; diarrhea. Killed.

Dog 19–17 (*Table III*).—Thin, long haired mongrel; weight 30.5 pounds.

Aug. 10, 1918. Simple obstruction made just above the ileocecal valve.

Aug. 11. Dog quiet. Shows slight distemper. Pulse and temperature normal.

Aug. 12. Weaker. Distemper somewhat worse. Laparotomy wound in good condition.

Aug. 13. Very dull. Vomits bile-stained mucus. Moderate diarrhea. Temperature 39.7°C. Killed at 10.40 a.m.

Autopsy.—Heart normal. Lungs show a few hyperemic areas over the surfaces and a reddening of the bronchial mucous membrane, but no definite pneu-

monia. The liver, spleen, and kidneys show moderate engorgement of blood. The intestine above the point of obstruction is moderately distended with slimy, putrid yellowish green fluid and gas. Its mucosa is hyperemic. The peritoneum is quite free and has its normal sheen.

Microscopic Examination.—Kidneys show a very slight degree of cloudy swelling. Otherwise entirely normal. Other organs likewise normal except for slight congestion of the smaller blood vessels.

The data given in the protocol of Dog 19–17 and in Table III serve to illustrate the second type of reaction to acute experimental obstruction; namely, that in which the symptoms of intoxication, including an increase in the blood urea, appear comparatively suddenly only a short time before death. The percentage of the test dye eliminated in this case was not decreased below the normal level until the last day when the most marked drop was recorded. So little dye was eliminated, in fact, that it could not be accurately estimated. Reference to the table shows that the rise in the blood urea was equally abrupt.

In this particular instance the resistance of the animal to the agent causing the intoxication was doubtless much lowered by the accompanying attack of distemper (*Bacillus bronchisepticus*) which had greatly weakened the dog before the reaction to the obstruction occurred. That an important part of the depression in the renal function was not due to the distemper may be inferred from the figures given in Table XII which show the absence of any definite impairment of kidney activity as measured by the ability to excrete urea and phenolsulfonephthalein in an uncomplicated case of distemper much more advanced than in this experiment.

Dog 19–19 (Tables IV and V).—Medium sized, short haired mongrel; weight 22.5 pounds.

Aug. 21, 1918. Simple obstruction made 10 cm. above ileocecal valve.

Aug. 22. Dog very active and bright. A small amount of milk and cracker meal eaten.

Aug. 23. Active and bright. No food given. Temperature 38.6°C. Pulse normal. Vomits.

Aug. 24. Slightly dull and weak. Pulse slightly weak. Temperature normal.

Aug. 25. Dog very dull. Vomits bile-stained mucus. Moderate diarrhea, late in day. Temperature 39.1°C. Pulse weak. Died during the night.

TABLE IV.

Dog 19–19. Simple Obstruction of the Ileum. Blood Urea. Phenolsulfonephthalein Elimination.

Date.	Weight.	Blood urea per 100 cc.	Phthalein elimination in 2 hrs.	Remarks.
1918	*lbs.*	*mg.*	*per cent*	
Aug. 18	20.4	28	69	No food previous 2 days.
" 19	20.4	27	68	
" 20	20.2	29	70	
Aug. 21	Simple obstruction 10 cm. above ileocecal valve.			
Aug. 22	20.0	28	69	Recovery from operation.
" 23	19.4	36	53	A few drops of urine lost.
" 24	19.0	38	66	Slightly dull.
" 25	18.4	93	32	Vomits bile-stained mucus. Fluid feces. Intoxication.

TABLE V.

Dog 19–19. Simple Obstruction of the Ileum.

Ratio: $\frac{\textit{Urea in 1 Hr.'s Urine}}{\textit{Urea in 100 Cc. of Blood}}$.

Date.	Hour.	Urea injected.	Urea excreted per hr.	Blood urea per 100 cc.	Ratio.	Remarks.
1918		*gm.*	*gm.*	*mg.*		
Aug. 18	8.15– 9.15		0.10	28	3.5	No food since Aug. 13.
	9.30–11.30	10	1.046	91	11.5	
Aug. 21	Simple obstruction 10 cm. above ileocecal valve.					
Aug. 25	1.45– 2.45		0.247	93	2.6	Dog very sick.
	3–5	10	0.734	165	4.4	

Autopsy.—Heart and lungs normal. Liver, spleen, kidneys, and mucosa of small intestine above obstruction moderately engorged with blood. Small intestine above obstruction distended with the typical slimy, putrid fluid and gas. No peritonitis.

Microscopic Examination.—Kidneys normal except for slight amount of congestion. Other organs also slightly congested.

The experiment presented in Tables IV and V illustrates the type of reaction in which the development of the intoxication is relatively precipitous, ending in death within 24 to 30 hours. The suddenness of the rise in blood urea and the fall in the percentage of test dye eliminated in the standard time is almost identical with that observed in the previous experiment (Table III). Dog 19–19 had no distemper and lived 1 day longer than Dog 19–17. This experiment serves as an additional check on the previous experiment which was complicated by the presence of distemper. The decrease in the urea ratio is rather striking here as in the first experiment.

TABLE VI.

Dog 18–119. Simple Obstruction of the Ileum. Blood Urea. Phenolsulfonephthalein Elimination.

Date.	Weight.	Blood urea per 100 cc.	Phthalein elimination in 2 hrs.	Remarks.
1918	*lbs.*	*mg.*	*per cent*	
Sept. 4	23.8	28	76	Fasted 3 previous days.
Sept. 5	Simple obstruction of ileum.			
Sept. 6	23.4	27	80	Recovery from operation satisfactory.
" 7	23.2	22	72	Dog bright and active.
" 8	22.7	10	67	
" 9–18	22–19	23–37	60–67	Dog apparently normal during this period. ½ pint of milk given on Sept. 18.
" 19	18.4	33	66	Showed first signs of intoxication. Dull. Vomited small amount. Temperature 39°C.
" 20	17.7	57	51	Weak and dull. Vomited more after eating small amount of milk. Pulse slightly weak.
" 21	17.4	81	48	Extremely dull and apathetic. Vomited. No diarrhea. Temperature 39.5°C.
" 22	17.0	124 140	22	Extremely dull and weak. Vomited. Tenesmus. Late in p.m. Temperature 37.4°C. Died during night.

Dog 18–119 (*Tables VI and VII*).—Small spaniel; weight 23 pounds. Has been employed several times previously for studies on proteose intoxication which will be reported in the following communication.

Sept. 5, 1918. Dog was bright and active. Temperature 39.1°C.

TABLE VII.

Dog 18–119. Simple Obstruction of the Ileum.

$$\text{Ratio: } \frac{\text{Urea in 1 Hr.'s Urine}}{\text{Urea in 100 Cc. of Blood}}.$$

Date.	Hour.	Urea injected.	Urea excreted per hr.	Blood urea per 100 cc.	Ratio.	Remarks.
1918		*gm.*	*gm.*	*mg.*		
Sept. 4	8.20– 9.20		0.123	28	4.4	
	9.30–11.30	10	0.917	71	12.9	Moderate diuresis.
Sept. 5	Simple obstruction of the ileum.					
Sept. 10	12.40– 1.40		0.119	28	4.2	Dog quiet after operation.
	1.50– 3.50	10	0.798	80	9.9	Moderate diuresis.
" 12	8–9		0.102	30	3.4	No food given.
	9.10–11.10	10	0.760	89	8.6	Mild diuresis.
" 19	12.50– 1.50		0.121	33	3.6	Small amount of milk ingested on previous day.
	2–4	10	0.613	112	5.5	Slight diuresis.
" 22	10.20–11.20		0.166	81	2.0	Dog very sick.
	11.30– 1.30	10	0.680	210	3.4	No diuresis. Extra fluid given.

Sept. 7. Still bright. Temperature 38.9°C.

Sept. 8. Slightly dull. Pulse slightly weak. Temperature 38.9°C.

Sept. 9 to 18. Bright but quiet. Pulse and temperature normal.

Sept. 18. Given ½ pint of whole milk containing lactose by stomach tube. Most of milk vomited.

Sept. 19. Acts dull. Vomits curdled milk and bile-stained mucus. Small mucus-coated stool. Temperature 39°C. Pulse slightly weak.

Sept. 20. Somewhat weaker and duller than on previous day. Vomits bile and watery mucus. Pulse weak.

Sept. 21. Extremely dull and apathetic. Vomits small quantity of mucus. No diarrhea. Temperature 39.5°C.

Sept. 22. Extremely dull and sick. Continues to vomit. Small hard mucus-coated stool. Pulse weak. Temperature at 6 p.m. 37.4°C. Died during the night. Vomitus and fluid feces in cage.

Autopsy.—Showed considerable emaciation. Heart and lungs normal. Peritoneal lining normal. Liver, spleen, and intestinal mucosa above point of obstruction are moderately hyperemic. Small intestine greatly distended with foul slimy fluid. Kidneys normal except for a slight grade of chronic pyelitis.

Microscopic Examination.—Moderate grade of chronic pyelitis. Remainder of kidney appears normal.

The protocol of Dog 18–119 presents a marked contrast to those given above in the length of time required for the intoxication to develop. The reason for this striking difference is not apparent from the data given unless we assume the possibility that a low grade tolerance has developed in the present case as a result of the previous injections of toxic proteose, in accordance with suggestions made in previous reports (17).

Table VI shows a relatively long period after the formation of the obstruction during which the blood urea and the elimination of phthalein remained normal. Following the ingestion of a very small amount of milk, most of which was vomited, the animal began to show symptoms of intoxication which continued to grow more intense until death 3 days later. As with Dog 18–138 (Tables I and II) there was found a gradual increase in the urea of the blood with a corresponding decrease in the amount of phthalein excreted.

Table VII shows an interesting change in the ratio

$$\frac{\text{Urea in 1 hr.'s urine}}{\text{Urea in 100 cc. of blood}}$$

both with and without the injection of urea. The difference between the normal value of the ratio before the obstruction and on the last day before death is very marked, especially when urea was injected. The decrease in the ratio appeared sooner than the fall in the phthalein output and was much more marked, as will be seen from inspection of both tables.

At autopsy a moderate grade of chronic pyelitis was found to be present although the remainder of the kidney both microscopically and grossly appeared normal. To what extent this condition was responsible for the subnormal activity of the kidneys cannot be said. However, since the process was found to be one of long standing while the impairment of function as measured by the tests was limited to the last 3 days, in which respect it corresponds very well with the previously described experiments, it seems probable that the impairment was due to the intoxication associated with the obstruction.

TABLE VIII.

Dog 19–55. Closed Loop of the Ileum. Acute and Chronic Intoxication. Blood Urea. Phenolsulfonephthalein Elimination.

Date.	Weight.	Blood urea per 100 cc.	Phthalein elimination in 2 hrs.	Remarks.
1918	*lbs.*	*mg.*	*per cent*	
Oct. 18–21	29.4	28–33	78–82	Oct. 19. Chloride excretion determined.
" 21	29.0	36	65	No food eaten. Drinks considerable water.
" 22	28.2	46	65	Refuses food. Very dull.
" 23	28.2	56	24	Dog very sick. Vomits. Diarrhea. Febrile reaction.
" 24	27.5	38	50	Still dull but brighter than on previous day.
" 25	27.2	44		Chloride excretion determined for 24 hrs. Dull. Refuses food.
" 26	27.2		54	Slightly improved.
" 27	27.0	33	55	Eats little food. Shows improvement.
Oct. 28–Dec. 3	24.5–26.5	28–40	55–67	Nov. 6. Chloride excretion determined. Dog practically normal during period
Dec. 4	26.4	45–78	34	47 cc. of proteose solution injected intravenously. Mild reaction.
" 5	24.2		54	Recovered.
" 9	23.5	54	66	Small dose of x-rays given.
" 10	23.2	44	65	Dog clinically intoxicated.
" 11	23.0	30	57	Slightly dull. Fluid feces. Vomited.
" 12	22.7	38	57	Condition the same.
" 13	22.5	58	54	Killed. Autopsy.

TABLE IX.

Dog 19–55. Closed Loop of the Ileum. Acute and Chronic Intoxication. Excretion of Injected Sodium Chloride.

Date.	Sodium chloride injected.	Chloride excretion over 24 hr. period.						Remarks.
		1st 2 hrs.	2nd 2 hrs.	3rd 2 hrs.	4th 2 hrs.	Remainder of 24 hrs.	Total 24 hrs.	
1918	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	
Oct. 19	6	1.88	1.29	0.84	0.61	1.96	6.58	2 days after loop operation—control. Dog bright and active.
" 25	6	1.02	0.58	0.41	0.32	0.83	3.16	Dog dull. Vomited. No food on previous day, or during present 24 hrs.
Nov. 6	6	1.89	1.42	0.94	0.62	2.69	7.56	Dog bright and active. Mixed diet given on previous day and during experiment by error.

Dog 19–55 (Tables VIII and IX).—Tall, thin setter, female; weight 29 pounds.

Oct. 17, 1918. Loop of ileum 30 cm. in length isolated and an anastomosis made to reestablish continuity of small intestine.

Oct. 18 to 21. Dog bright and active. Pulse and temperature normal.

Oct. 21. Slightly dull. Vomits food and mucus. Passes soft feces. Pulse normal. Temperature 38.9°C.

Oct. 22. Dull. Vomits small amount of mucus.

Oct. 23. Much more dull. Acts sick. Temperature 38.8°C. Vomits bile-stained watery mucus. Passes soft and semifluid feces.

Oct. 24. Still dull but slightly brighter than on previous day. Vomits small amount of foamy, bile-stained mucus. No diarrhea. Pulse and temperature about normal.

Oct. 25. Dog dull and weak. Vomits. Passed soft feces.

Oct. 26. Quiet but somewhat improved. No vomiting or diarrhea. Pulse and temperature normal.

Oct. 27. Vomited small amount. Fluid feces.

Oct. 28 to Dec. 3. Dog was quiet and acted weak but was moderately bright. Ate a small amount of food occasionally. Weight varied from day to day between 24.5 and 26.5 pounds. Blood urea varied from 16 to 40 mg. per 100 cc., and 2 hour phthalein elimination varied between 55 and 67 per cent.

Dec. 3. Slight distension of abdomen with faint peristaltic waves in loop observed. Dog slightly dull.

Dec. 4. 1.75 cc. of toxic proteose solution per pound of body weight given intravenously; this produced a mild grade of intoxication. Recovery from this was comparatively rapid.

Dec. 9. Distension of abdomen somewhat increased and peristalsis more distinct. At 5.30 p.m. given 190 milliampere minutes x-rays over abdomen in three doses.[1]

Dec. 10. Dog shows greater weakness and dullness than on previous day. Vomited bile-stained mucus and food. Moderate diarrhea.

Dec. 11. Brighter but very weak and quiet.

Dec. 12. Greater dullness. Pulse weak.

Dec. 13. Very dull and weak. Vomited; diarrhea. 11.30 a.m. Killed.

Autopsy.—Heart and lungs negative. Liver and spleen showed a moderate degree of atrophy. Pancreas normal. Kidneys normal except for pallor of the inner cortical zone due to the deposition of fat. Peritoneum clean and glistening. The isolated loop of ileum enormously distended with semifluid, slimy, greenish gray material with a fleshy, slightly putrid odor. The muscular coat was greatly hypertrophied. The mucosa of the loop was smooth and velvety in appearance with no visible signs of ulceration or necrosis.

Microscopic Examination.—Kidneys normal.

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[1] Detailed statement to be given in a subsequent report.

Table VIII with the protocol of Dog 19–55 presents some interesting facts. A 30 cm. closed loop of the lower portion of the ileum was isolated in the manner described elsewhere (17) by making two sections of the intestine, turning in the cut ends, and reestablishing the alimentary canal by a simple lateral anastomosis between the remaining portions. The animal recovered satisfactorily from the immediate effects of the operation and remained active and bright until the 4th day when symptoms of intoxication began to appear.

These symptoms became gradually more marked until the 6th day, after which they gradually decreased in intensity until recovery was apparently complete on the 8th day. The cause of this reaction was possibly not the presence of the isolated closed loop but a temporary stasis above the point of anastomosis, since the loop itself showed no enlargement or abnormal peristalsis typical of a distended loop. However, it cannot be denied that this may have been a loop intoxication followed by improvement due to established tolerance.

During this period the blood urea increased considerably above normal. The phthalein elimination showed the usual downward movement on the day of the most intense intoxication and remained slightly below normal as long as the symptoms of intoxication lasted. The blood urea likewise remained above the normal level for a fasting dog until recovery.

Table IX shows that the capacity of the kidneys to eliminate injected sodium chloride was also lowered to a moderate degree at this time and then returned to normal upon recovery.

From this date the dog remained practically normal for over a month, taking food daily and living without apparent inconvenience from the isolated loop. On December 4, 1.75 cc. of toxic proteose solution per pound of body weight were given intravenously. Only a mild grade of intoxication was produced, although, as will be seen from Table VIII, the blood urea on that day rose from 45 to 78 mg. per 100 cc. following the injection and the phthalein elimination dropped from 67 per cent on the preceding day to 34 per cent during the intoxication.

The intensity of the reaction due to this dose of the proteose preparation was appreciably less in this case than in the case of a normal dog (Dog 19–59). A slightly larger dose, in fact, *i.e.* 2 cc. per pound

of body weight, was shown to be the lethal dose for a normal dog (Dog 19–29) in another experiment. There appears, then, to have been a slightly increased resistance to proteose poisoning in the case of Dog 19–55 which suggests to us the possibility of this dog's having acquired a partial immunity to such intoxication following this subacute loop intoxication.

The x-ray treatment constitutes part of another experiment, which will be reported at another time.

TABLE X.

Dog 19–42. Simple Obstruction of the Lower Portion of the Jejunum. Blood Urea. Phenolsulfonephthalein Elimination.

Date.	Weight.	Blood urea per 100 cc.	Phthalein elimination in 2 hrs.	Remarks.
1918	*lbs.*	*mg.*	*per cent*	
Oct. 10	27.0	29	70	No food on previous day.
" 31	27.6	30	70	Food given on previous day.
Nov. 1	Simple obstruction at about middle of small intestine.			
Nov. 2	27.5	28	72	Recovery from operation.
" 3	27.2	21	70	Bright and active.
" 4	26.8	25	39	Acts very dull.
" 5	26.4	39	68	Drinks water but refuses food. Dull.
" 6	26.0	48		Chloride excretion determined (Table XI). Dull and weak. Vomits bile-stained mucus.
" 7	25.2	58	45	Duller than on previous day. Vomiting increased.
" 8	24.0	50	32	Condition unchanged.
" 9	23.2	64	-	Chloride excretion determined (Table XI). Very dull and sick.

Dog 19–42 (*Tables X and XI*).—Medium sized collie, adult female; weight 27 pounds.

Oct. 6, 1918. Dog fasted 3 previous days. Given 5 gm. of sodium chloride and 10 gm. of urea dissolved in 250 cc. of distilled water intravenously. Chloride excretion was then followed for the next 24 hours in three subperiods; *viz.*, 1st hour, next 2 hours, and remainder of 24 hour period.

Nov. 1. Simple obstruction produced at about the middle of small intestine in the usual way.

Nov. 2. Dog is quiet but not dull. Temperature 39°C.

TABLE XI.

Dog 19–42. Simple Obstruction of the Lower Portion of the Jejunum. Excretion of Injected Sodium Chloride.

Date.	Sodium chloride injected.	Chloride excretion over 24 hr. period.				Remarks.
		1st hr.	Next 2 hrs.	Remainder of 24 hrs.	Total 24 hrs.	
1918	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	
Oct. 6	5.0	0.822	0.841	3.327	4.989	Fasted 3 previous days. Active and bright.
Nov. 1	Simple obstruction at middle of small intestine.					
Nov. 6	5.0	0.395	0.412	1.180	1.987	
" 9	5.0	0.105	0.176	0.233	0.514	Total period about 22 hrs. instead of 24.

Nov. 3. Much brighter. Normal pulse and temperature.

Nov. 4. Acts very dull, lying down most of the time. Small amount of pus in wound. Pulse slightly weak. Temperature normal.

Nov. 5. Still dull. Drinks water but refuses food. Pulse slightly weak.

Nov. 6. Dull and weak. Vomited small quantity of bile-stained mucus. No diarrhea. Pulse weak.

Nov. 7. Condition unchanged.

Nov. 8. More dull. Vomiting increased. Clinically sick. Temperature 38.9°C.

Nov. 9. Very dull and sick. Abdomen moderately distended. Visible peristaltic waves. Vomited considerable clear, stringy, bile-stained mucus. Hard feces coated with mucus. Pulse slightly weak and irregular.

Nov. 10. Dog died at about 6 a.m. Fresh vomitus and fluid feces in cage.

Autopsy.—Body still warm. Rigor mortis incomplete. Wound showed slight amount of suppuration, but mostly healed. No peritonitis. Heart and lungs normal. Spleen and liver irregularly engorged with unclotted blood. Kidneys negative except for slight pallor of inner zone of cortex due to deposition of fat. Pancreas negative. Small intestine above point of obstruction greatly distended with typical semifluid, yellowish green material with a foul odor. The mucosa is irregularly hyperemic but is not ulcerated. The portion of intestine below the obstruction is contracted but has a normal appearance.

Microscopic Examination.—Kidneys entirely normal. Other organs likewise normal.

Table X (Dog 19–42) shows a gradual increase in the urea content of the blood and a corresponding decrease in the percentage of

phthalein eliminated from day to day following the production of a simple obstruction of the jejunum.

Table XI gives the rate of excretion of injected sodium chloride over a 24 hour period before the operation, on the 5th day afterwards, and again on the last day before the animal died. Inspection of the figures recorded on the various days shows a marked diminution in the quantity of salt eliminated. Between one-half and one-third only of the normal amount of total chlorides was excreted on the 5th day and only slightly less than one-tenth of the normal quantity appeared in the urine of the last day. The degree of retention of chlorides during the intoxication appears to be much greater, therefore, than that of urea and phenolsulfonephthalein.

In this case as in all the above with the exception of Dog 18–119 (Tables VI and VII) the kidneys appeared normal both grossly and microscopically, in spite of the terminal impairment of renal function.

TABLE XII.

Dog 19–24. Distemper (B. bronchisepticus).

Ratio: $\frac{\textit{Urea in 1 Hr.'s Urine}}{\textit{Urea in 100 Cc. of Blood}}$.

Blood Urea. Phenolsulfonephthalein Elimination.

Date.	Hour.	Urea injected.	Urea excreted per hr.	Blood urea per 100 cc.	Ratio.	Phthalcin elimination in 2 hrs.	Remarks.
1918		*gm.*	*gm.*	*mg.*		*per cent*	
Sept. 5	8–9		0.131	29	4.6		
	9.10–10.10	10	0.720	83	8.69	68	Dog active and bright. Moderate diuresis after urea injection.
	10.10–11.10		0.659	81	8.16		
Sept. 8	Develops distemper.						
Sept. 19	8.50– 9.50		0.174	41	4.2		
	10–11	10	0.727	94	7.7	60	Dog in moribund condition. Blood pressure extremely low. Moderate diuresis after urea injection.
	11–12		0.658	92	7.1		

Dog 19–24 (*Table XII*).—Large Scotch collie, female; weight 30.5 pounds.

Sept. 5, 1918. At the end of 3 days fast urea ratio determined after injection of 10 gm. of urea (Table XII). 2 hour phthalein elimination 68 per cent.

Sept. 8. Began to show signs of distemper (*B. bronchisepticus* infection). Acted slightly dull. Sneezed occasionally. Slight amount of exudate about nares and eyes.

Sept. 10. Frank distemper. Dull. Temperature high. Increased exudate about nares and conjunctivæ. Weight 27.5 pounds.

Sept. 18. Condition much worse. Very weak and apathetic. Pulse extremely weak. Lies in cage twitching and jerking. Nares and eyes covered with mucopurulent exudate. Weight 23.5 pounds. Phthalein elimination for 2 hours, 61 per cent. Blood urea 42 mg. per 100 cc.

Sept. 19. Dog moribund. Condition of previous day aggravated considerably. Dye elimination 60 per cent. Blood urea 41 mg. 3 p.m. Killed.

Autopsy.—Heart normal. Lungs show diffuse patches of hyperemia and edema. Bronchial passages very hyperemic. Bronchial exudate mucopurulent. Lymph nodes hypertrophied. Liver, spleen, kidneys, pancreas, and gastrointestinal tract normal.

Microscopic Examination.—Kidneys and liver show slight degree of cloudy swelling, but are otherwise normal. Lungs show engorgement and edema. Other organs normal.

The last experiment of the present series on a dog with uncomplicated distemper is included as a control for one of the foregoing numbers in particular (Table III), in which the animal developed a moderate case of distemper after the operation for the production of the obstruction. It serves for a check also on the other experiments because of the common factor of low blood pressure in both conditions, which, if not controlled, might invalidate any study on renal function. The severity of the infection was much more extreme in the present case than in that of Dog 19–17, and the fall in blood pressure was much greater than is often observed in the intoxication of intestinal obstruction except in the last hours after the animal has passed into a state of shock.

Table XII shows the non-influence of the most advanced stage of distemper upon the urea ratio and the phenolsulfonephthalein elimination. Although the blood pressure was extremely low during the distemper period, the urea ratio and the percentage of dye excreted were only very slightly below the normal. This fact justifies the assumption, therefore, that the marked retention of urea, phthalein,

and chlorides during the period of intoxication following obstruction of the small intestine is only in very small part, if at all, due to the factor of lowered blood pressure.

DISCUSSION.

The experiments presented in the above tables definitely clear up the question set forth in the early part of this paper with reference to the efficiency of the kidneys during the intoxication of acute intestinal obstruction. The excretory function is decidedly impaired in this condition.

Individuals with intestinal obstruction show a heaping up of all non-protein nitrogenous substances in the blood. Urea is most conspicuous in this material. The kidney is evidently unable to secrete any of these nitrogenous substances with its normal facility. These substances are being formed with abnormal speed, so there is a great accumulation in the blood and tissues. The kidney in this condition reacts much like the kidney of chronic nephritis, although there is no anatomical injury and the kidney of intestinal obstruction is only temporarily insufficient. With relief of the obstruction and clinical recovery the kidney function returns to normal. This injury can be repaired easily and leaves no trace behind, in as far as modern histological methods can show.

The decrease in output of fluid in the urine is due in part to the loss of fluid by vomiting, but when fluids are given intravenously the lack of diuresis may well be explained by the injury of renal epithelium. It has long been known that certain proteoses inhibit the flow of urine, but the experiments which demonstrated this were performed before the day of kidney function tests.

That dogs with very severe distemper and bronchopneumonia show little if any drop in renal function is somewhat surprising. It is known that these animals at times show a high non-protein nitrogen, but never approaching the high figures of intestinal obstruction. The dogs also show a great rise in the basal urinary nitrogen excretion, indicating a considerable breaking down of body protein. These facts demonstrate a clear-cut difference between the intoxications of pneumonia and intestinal obstruction in dogs.

We wish to recall three important facts concerning the intoxication of intestinal obstruction: (1) There is a great increase in the elimination of urinary nitrogen, which is dependent upon the intoxication. (2) There is a great increase in the non-protein elements of the blood. These two facts indicate cell injury. (3) There is a decrease in kidney excretory function which is most clearly shown by the inability of the kidney to secrete the normal amounts of urea, sodium chloride, and phenolsulfonephthalein.

The last fact is most important in establishing beyond reasonable doubt the presence of some poison in the blood stream. These experiments taken together with those outlined in the next paper indicate again that the same poison is also present in the lumen of the obstructed intestine. Some may insist upon the isolation of some poison from the blood stream, but with our present methods that proof cannot be given. In fact the whole blood is non-toxic, but this is no proof that no poison exists. A lethal dose of proteose will disappear from the blood stream within 3 to 5 minutes and yet will work its fatal reaction which may require 6 to 8 hours or longer for its completion.

We believe in picturing this reaction in the kidneys as a part of the general cell protein injury which results from the presence of the obstructed intestine. The poison, let us say, acts directly upon the epithelium of the kidney and causes temporary paralysis or impairment of its secretory function. There is no histological evidence of any cell injury, but we realize that function may be impaired without any definite change in structure. Repair of this injury may be effected within 24 to 48 hours after clinical recovery from the intoxication.

In the treatment of this condition no physician can afford to ignore this established fact that a definite impairment of kidney function develops as a part of the intoxication of intestinal obstruction. The two conditions usually parallel each other closely. The degree of intoxication which may develop in ileus is sometimes hard to evaluate clinically. We suggest that the non-protein nitrogen or urea nitrogen of the blood, as well as the renal function, may give warning of a grave intoxication which may be masked clinically. We are aware that ileus may persist with stormy symptoms for many days without really grave intoxication. Again, the condition may appear to be mild

clinically yet associated with high blood urea and a low renal function. In the last instance there should be no doubt of a serious intoxication and the necessity of urgent measures.

SUMMARY AND CONCLUSIONS.

Associated with the intoxication of intestinal obstruction there exists a definite impairment of the excretory function of the kidneys.

The degree of functional depression corresponds roughly with the intensity of the clinical intoxication.

The decrease in the urea ratio and in the capacity of the kidneys to excrete sodium chloride is more marked than is the percentage decrease of phenolsulfonephthalein elimination.

The great increase in the non-protein nitrogen of the blood usually observed in acute intestinal obstruction, which has hitherto been explained as being due entirely to an increased rate of protein catabolism, is due in part to retention of the products released from the injured cell protein.

It is probable that the impaired renal function is due to direct action of the toxic substances upon the renal epithelium.

The actual demonstration of this renal injury is perhaps the strongest evidence so far obtained to prove the presence of an actual toxic substance in the blood during intestinal obstruction.

This obscure disability of the kidneys during the height of the intoxication of acute ileus should always be considered in the clinical management of this condition. It may also serve as a guide to indicate the degree of intoxication.

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II. RENAL FUNCTION INFLUENCED BY PROTEOSE INTOXICATION.

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In the preceding paper (1) we reported a number of experiments which showed that the excretory function of the kidneys is definitely impaired during the intoxication of intestinal obstruction. Since most of the animals employed in that study died late during the night, the maximum degree of impairment was not always ascertained by the tests made on the previous day. This fact together with a number of other considerations made it highly desirable that the study should be continued under somewhat different conditions in which the reaction could be better controlled.

It has been shown (2–6) that the intravenous injection of the toxic proteose material isolated from the contents of the obstructed small intestine produces symptoms closely resembling, if not identical with, those characterizing the intoxication of acute ileus. Therefore, we decided to employ this more simple method of inducing the intoxication for a supplementary study of the renal function. In this way the time and the intensity of the reaction can be regulated at will for various experiments. Such a study should at the same time supply further evidence for or against the contention that the intoxication of acute intestinal obstruction is a proteose intoxication as has been suggested by Whipple, Stone, and Bernheim (2–5) and Whipple, Rodenbaugh, and Kilgore (6).

It has already been satisfactorily determined that no anatomical lesion of the kidneys is produced by the parenteral injection of the toxic proteose material. No histological alterations have ever been observed in the kidneys of normal dogs given fatal doses in this laboratory. In the anatomical laboratory of this University, furthermore, rats were stained *intra vitam* with Niagara blue, a relatively non-toxic vital dye, after having received intraperitoneally a series of large sublethal doses of toxic proteose. Although every grade of intoxication was produced in these animals, no definite changes in the structure of the kidneys that could be associated with the intoxication were demonstrated even by this delicate method of detecting cell injury.[1] However, this does not necessarily mean that there may not have been even a profound temporary impairment of renal

[1] McQuarrie, I., unpublished experiments.

function, since it was shown in the preceding paper that a definite impairment exists during the intoxication of intestinal obstruction in spite of the absence of anatomically demonstrable changes in the kidney.

The rapid rise in the non-protein nitrogen of the blood often to a very high level, observed (7) to follow the injection of toxic proteose in a fasting animal suggests inefficient activity on the part of the kidneys or unusually rapid destruction of body protein. That there is a greatly increased protein catabolism has been satisfactorily demonstrated by experiments (8) which showed a marked increase in the total urinary nitrogen excretion to follow the injection of a large dose of toxic proteose. A curious fact about this increase in total urinary nitrogen is that the apex of the curve of excretion occurs almost invariably during the second 24 hours after the injection of the poison, while the blood non-protein nitrogen reaches its maximum level on the 1st day shortly after the clinical signs of intoxication are most manifest. No entirely satisfactory explanation for this peculiar delay has been offered, although it suggests either a partial delay in the process of tissue disintegration or a transient impairment of kidney function.

Methods.

Dogs, chiefly females, were used here as in the previous experiments because they react characteristically to proteose intoxication and are easily bled and catheterized. Only healthy young adult animals were selected after renal function tests showed them to possess normal kidneys.

The various methods employed for measuring the kidney function in the experiments described in the preceding communication were applied in the present work also; namely, (1) that of determining the urea-excreting power of the kidneys; (2) that of determining the rate of excretion of injected sodium chloride; (3) the phenolsulfonephthalein elimination method of Rowntree and Geraghty (9, 10).

The last two methods were employed here in exactly the same manner as in the previous work. The first method, that of measuring the urea-excreting capacity of the kidneys, was modified somewhat in the experiments to be described here. In addition to determining the ratio

$$\frac{\text{Urea in 1 hr.'s urine}}{\text{Urea in 100 cc. of blood}}$$

after the injection of urea intravenously, we determined it without the urea administration in a number of instances. In still other

cases the rate of excretion of injected urea over a 5 hour period was determined before and again after the injection of the toxic proteose preparation without reference to the blood urea. Wherever the ratio was determined, the blood sample was taken as nearly in the middle of the hour as possible.

The data obtained on each dog with regard to the renal function at the time of selection serve as a control for the later experiments on that particular animal, since the same general conditions obtained in both periods.

After this preliminary examination the dog was given a dose of the toxic material isolated from the contents of an obstructed intestine. From 1 to 3 hours after this injection the different renal function tests were applied as in the control period.

The poisonous material used in this study for producing the intoxication is isolated from the contents of the experimentally obstructed intestine of the dog and from human autopsy material in the following manner. The crude semifluid product is centrifugalized at high speed until all the coarser particles have been thrown to the bottom of the tube. The toxic fraction is then precipitated from the supernatant fluid by the addition of five volumes of 95 per cent alcohol. The flocculent alcoholic precipitate is separated by centrifugalization from the alcoholic mixture, after which it is redissolved in a volume of distilled water equal to three times that of the original obstruction fluid material. This solution which always contains a considerable quantity of albuminous material is made faintly acid with acetic acid and brought to the boiling point in a water bath. The precipitated proteins are filtered off, leaving a clear, slightly opalescent solution containing the toxic proteoses.

As has been pointed out elsewhere by Whipple and Van Slyke (11), the chemistry of the proteose group of substances is not at all clearly understood and what is spoken of in the present paper as a "proteose solution" is simply an aqueous solution of the alcoholic precipitate obtained in the manner described above, from which the albuminous material has been removed. Nevertheless, the unusual substance or group of substances isolated in this way from the mixture contained in the lumen of the obstructed small intestine gives the symptom complex characteristic of acute obstruction when injected

intravenously in the dog and, therefore, serves our present purposes very well.

A few other proteoses from various sources have also been used to supplement the experiments performed with the poisonous material already described. The object of including them in the present study was to ascertain to what extent the impairment of renal function is a property of all proteoses or to see if any other proteoses behave in this way.

Proteoses were obtained from the following sources: (1) commercial Witte's "Peptone," which consists chiefly of proteoses; (2) pepsin digest of liver (Martin's broth); (3) pepsin digest of sterile muscle tissue; (4) pepsin digest of muscle tissue accidentally contaminated with proteolytic bacteria, some of which were found to be anaerobes; (5) sterile muscle tissue digested by three different types of proteolytic anaerobic bacteria separately in pure culture. The three organisms used for the last digestion method were *Bacillus histolyticus*, *Bacillus sporogenes*, and *Bacillus bifermentans*.[2]

Material from each of the several protein digests was injected from time to time at different stages of the digestion, beginning on the 1st day in nearly all cases and continuing as long as 3 weeks in some. Chemical tests for determining the approximate yield of proteoses were made from time to time. In all cases the supernatant fluid from the digests was first filtered to remove larger particles. The filtrate was then heated for 20 minutes over a boiling water bath for sterilization and again filtered before it was injected. In the only case in which marked symptoms of intoxication were produced by this preliminary injection, namely that of the digest accidentally contaminated with proteolytic bacteria, the toxic mixture was treated in a manner exactly like that described above for the isolation of the toxic proteose from intestinal obstruction material.

From $1\frac{1}{2}$ to 3 hours after the injection of the proteose preparation in each instance, the blood urea was determined and the phenolsulfonephthalein elimination test was performed.

[2] These three organisms were suggested to us by Dr. K. F. Meyer and furnished in pure culture by Mrs. H. H. Heller.

In the experiments which follow, the same general methods of administering test substances, of collecting blood and urine samples, and of quantitating the urea, chlorides, and phthalein were used as those described in the preceding communication with a few minor differences. For example, in one short series of experiments the dye was given intravenously and in another set subcutaneously instead of intramuscularly. It was thought that the use of a variety of routes for the dye might afford some evidence regarding the part played by absorption in the failure of the intoxicated animal to eliminate it promptly.

In order better to control the extrarenal factor of loss of fluid from the blood to the tissues and to the alimentary tract during the period of intoxication, comparatively large amounts of fluid were given either by stomach tube when vomiting was slight or intravenously when the animal vomited frequently. Isotonic saline or glucose solution and in a few cases acacia solution were employed for this purpose and in every instance more fluid was given during the period of intoxication than during the corresponding control period.

EXPERIMENTAL OBSERVATIONS.

In most of the following tables several like experiments have been grouped together in the same table in order that they may be easily compared and allow a saving of space. It will be observed, too, that the various grades of intoxication produced are spoken of as "slight," "mild," "moderate," and "intense." There is no very well defined line of demarkation between these, but by a "slight" intoxication is meant a definite, though transient febrile reaction, nausea with a slight amount of vomiting of mucus which is not necessarily bile-stained, usually a slight amount of tenesmus without definite diarrhea, and always a slight dullness. A "mild" intoxication is a somewhat more noticeable upset in which the rectal temperature may reach 40°C. and there is occasional vomiting of foamy, stringy, usually bile-stained mucus some time during the first 2 hours with more marked tenesmus and a soft or semifluid stool and more definite dullness. By a "moderate" grade of intoxication is meant a reaction still more marked in which the temperature may rise above 41°C.

and remain above normal for a number of hours; the blood pressure falls slightly below normal; there is intermittent vomiting of bile-stained mucus over several hours and frequent passage of small, soft and then semifluid stools, and considerable tenesmus. The animal appears dull and somewhat weak for a number of hours and often remains slightly dull even on the following day. An "intense" reaction following the injection of a lethal or slightly sublethal dose of the toxic substance is usually one in which there is frequent vomiting over the first few hours and diarrhea usually lasting longer, lowering of blood pressure, an early rise in temperature far above the normal with a later fall to a subnormal level. As the intestines are emptied the character of the excreta changes from the hard normal stool to a soft and finally a semifluid consistency. When a large portion of this has been evacuated, flakes or casts of mucus and epithelium from the intestinal mucosa begin to appear. Following this there is often found a small amount of clear, watery mucus which in the fatal cases is usually blood-stained. At this stage the body temperature may be considerably below normal and the sphincter ani is relaxed, allowing the watery excreta to ooze out slowly. The animal may show great prostration just before this final collapse or may die in convulsions. While this is the usual picture when the intoxication is prolonged, a large dose of the poison may cause death within an hour or two with little or no vomiting or diarrhea. In the more severe grades of intoxication there is nearly always a rapid rise in the non-protein nitrogen of the blood which runs parallel in a general way with the intensity of the intoxication.

Table I represents four short experiments in which the rate of excretion of injected urea was determined before and again after the injection of a sublethal dose of toxic proteose from a human autopsy which produced in each case a moderate grade of intoxication. The dye was injected subcutaneously at the same time that the urea was given intravenously.

These experiments uniformly show a moderate decrease in the amount of both urea and phthalein eliminated after the proteose injection. With the dye it will be noted that the 1st hour excretion suffers proportionately a decidedly greater decrease below the normal than the 2nd hour. This is doubtless due in part to the greater

TABLE I.

Injection of Proteose. Excretion of Injected Urea and Phenolsulfonephthalein.

	Proteose injected.	Urea injected.	Urea excretion.							Phthalein elimination.			Remarks.
			Fore-period of 1 hr.	Length of time after urea injection.									
				1st hr.	2nd hr.	3rd hr.	4th hr.	5th hr.	Total.	1st hr.	2nd hr.	Total for 2 hrs.	
1918	*cc.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*per cent*	*per cent*	*per cent*	
Dog 18–2, weight 49 lbs.													
Feb. 22	0	25	0.19	2.30	2.11	1.60	1.35	1.13	8.68	47	26	73	Marked diuresis.
" 28	98	25	0.19	1.46	1.21	1.07	0.88	0.73	5.54	31	20	51	Urea and dye injected 1 hr. 10 min. after proteose. Moderate intoxication.
Dog 18–3, weight 36 lbs.													
Feb. 20	0	18	0.17	1.92	1.58	1.33	1.20	1.05	7.25	47	30	77	Marked diuresis.
Mar. 5	72	18	0.18	1.36	0.92	0.93	0.81	0.63	4.83	25	21	46	Urea and dye injected 1½ hrs. after proteose. Intoxication only mild.
Dog 18–85, weight 19 lbs.													
Mar. 2	0	12	0.20	1.62	1.25	1.03	0.75	0.61	5.46	54	23	77	Marked diuresis.
" 10	37	12	0.18	0.87	0.78	0.54	0.47	0.37	3.21	31	23	54	Urea and dye injected 1¼ hrs. after proteose. Moderate intoxication.
Dog 17–227, weight 40 lbs.													
Mar. 19	0	15.5	0.17	1.56	1.20	1.08	0.94	0.81	5.76	47	25	72	Considerable diuresis.
Apr. 1	80	15.5	0.16	1.26	1.07	0.50	0.25	0.20	3.44	28	21	49	Urea and dye injected 1½ hrs. after proteose. Moderate intoxication.

relative concentration of dye in the blood stream during the 2nd hour in the case of the intoxication period, but it may be due in part also to slower absorption of the dye under these circumstances.

Although not given in Table I the volume of urine excreted during some of the later hours of the period of intoxication was occasionally slightly greater than that of the corresponding hours of the control period, due perhaps, in some cases at least, to the increased amount of water taken by the dog after the most acute stage of the intoxication had passed. Yet, there is in these instances, also, a considerable decrease in the amount of urea excreted, which seems to show that extrarenal factors, such as a lessened availability of water to be secreted, alone are not responsible for the failure of the animal to eliminate the normal quantity of administered urea during the intoxication.

Dog 18–2 (*Table II*).—

Autopsy.—Performed immediately after death. Heart distended with dark, prune-colored blood which coagulates very slowly. Numerous subendocardial petechial hemorrhages in ventricles. Lungs normal. Liver and spleen intensely engorged. Kidneys negative except for marked congestion. Intestinal mucosa very intensely engorged with blood. Considerable blood in lumen of intestines.

Microscopic Examination.—Shows nothing except congestion of all the viscera including the kidneys.

Table II shows the effect of various grades of proteose intoxications upon the ratio:

$$\frac{\text{Urea in 1 hr.'s urine}}{\text{Urea in 100 cc. of blood}}$$

when no urea is administered and upon the elimination of phenolsulfonephthalein.

It will be seen from these experiments that there is a lowering of the urea ratio and an almost parallel reduction in the amount of dye eliminated, both of which correspond with the grade of intoxication. Although in the cases of less intense intoxication the absolute amount of urea excreted per hour is slightly increased as the blood urea rises, the increase does not keep pace with this rise and consequently the ratio falls. This indicates a deficiency in the response of the kidney to even a small load. As will be seen in the control periods of some

TABLE II.

Injection of Proteose. Effect upon the Ratio $\frac{\textit{Urea in 1 Hr.'s Urine}}{\textit{Urea in 100 Cc. of Blood}}$ *and the Elimination of Phenolsulfonephthalein. No Urea Injected.*

	Condition of experiment.	Hour.	Urea excreted.	Blood urea per 100 cc.	Urea ratio.	Phthalein elimination in 2 hrs.	Remarks.
1918			*gm.*	*mg.*		*per cent*	
Dog 18–2, weight 49 lbs.							
Apr. 20	Control.	9–10	0.124	28	4.43		300 cc. of water by stomach tube ½ hr. before phthalein.
		10–11	0.105	25	4.20	74	
		11–12	0.117	26	4.50		
" 29	Intense proteose intoxication.	11.30–12.30	0.062	44	1.41		10.40 a.m. 96 cc. of proteose injected. 12.30 p.m. Dye injected. 300 cc. of water by tube 11.50 a.m., partly vomited.
		12.30– 1.30	0.052	48	1.08	Mere trace.	12.50 p.m. 200 cc. of $\frac{M}{6}$ salt solution intravenously.
		1.30– 2.30	0.043	52	0.82		Marked oliguria. Apr. 30. Dog died from the intoxication. Blood urea 90 mg.
Dog 18–119, weight 24 lbs.							
July 8	Control.	9.45–10.45	0.271	34	7.60	71*	10.15 a.m. Dye injected.
	Mild intoxication.	1–2	0.183	40	4.50	57*	11 a.m. Proteose injected. 12.30 p.m. 250 cc. of water. 1.30 p.m. Dye injected.

* Dye given intravenously—30 minute collection.

TABLE II—*Concluded.*

	Condition of experiment.	Hour.	Urea excreted.	Blood urea per 100 cc.	Urea ratio.	Phthalein elimination in 2 hrs.	Remarks.
1918			*gm.*	*mg.*		*per cent*	
July 20	Control.	9–10	0.122	27	4.43	70*	
	Moderate intoxication.	12.30– 1.30	0.146	41	3.54		10.20 a.m. 150 cc. of dilute proteose solution.
		2.32– 3.32	0.128	57	2.25	41*	1.35 p.m. 200 cc. of 5% acacia solution intravenously.
		8–9 next day.	0.146	43	3.4	58*	2.20 p.m. Dye injected. 2nd sample of blood at end instead of middle of hr. as usual. Dog slightly dull next day.
" 24	Control.	10–11	0.145	36	4.0		
	Very slight intoxication.	1–2	0.160	44	3.6		12 m. 23 cc. of dilute proteose solution.
		2–3	0.200	38	5.2	62*	3.15 p.m. Dye injected.
Dog 19–59, weight 49 lbs.							
July 10	Control.	10–11	0.234	38	6.14	72*	
	Moderate intoxication.	3–4	0.239	67	3.47	50*	11.30 a.m. 400 cc. of very dilute proteose. 4.15 p.m. Dye in 100 cc. of water.

of the following experiments (Tables III and IV), the normal kidney responds to an increase in the blood urea by producing a rise in the ratio and never a fall, as in this case.

Dog 18–2 with an intense grade of intoxication shows even a decrease in the quantity of urea excreted per hour although the blood urea has increased to 100 per cent above its normal value in a fasting dog. The ratio has decreased from one-fifth to one-third of its value before the proteose injection. The amount of dye eliminated in this 3 hour period was too small for accurate estimation by the colori-

metric method. There was marked oliguria in spite of the fact that a large quantity of fluid was given intravenously and by mouth.

It will be noted that, although the dye was given intravenously in the majority of the experiments shown in the table, the percentage eliminated was decreased in these as in other cases and that the decrease corresponded with the grade of intoxication. This observation proves that the factor of delayed absorption of the dye does not account for the diminution in the quantity excreted during the intoxication.

Dog 17–227 (Table III).—

Autopsy.—Heart and lungs negative. Liver markedly congested. Spleen intensely engorged with blood. Large subcapsular hemorrhages bulge from surface. Kidneys greatly congested. Otherwise normal. Gastrointestinal tract negative except for most intense engorgement of blood capillaries in mucosa of jejunum, duodenum, and rectum with less marked congestion in other regions.

Microscopic Examination.—Kidneys normal except for congestion. Other viscera also congested.

Tables III and IV include four experiments in which the urea ratio and phenolsulfonephthalein elimination were determined before and again after injection of various doses of toxic proteose. In each case except that of Dog 18–59 a 1 hour control period preceded the injection of urea and in only one case, that of Dog 17–227, did this control hour come after the injection of the poison. The data obtained for the fore-hour in the larger control period can be taken as a baseline from which to estimate more accurately the increase in the urea excreted per hour and in the blood urea due to the injection of urea alone. A comparison of these values with those obtained after the proteose injection shows the changes due to the proteose alone.

The decrease in the ratio in these experiments is very striking and is considerably greater than that found in the experiments presented in Table II in which no urea was injected. The reduction in the rate of phthalein elimination corresponds more nearly with the decrease in the urea-excreting power as measured by the methods used in the previous experiments. The apparent lowering of renal efficiency here as in the previous experiments corresponds in a general way with the intensity of the intoxication.

TABLE III.

Injection of Proteose. Effect upon the Ratio $\frac{\textit{Urea in 1 Hr.'s Urine}}{\textit{Urea in 100 Cc. of Blood}}$ *and the Elimination of Phenolsulfonephthalein. Urea Injected.*

	Proteose injected.	Urea injected.	Hour.	Urea excreted.	Blood urea per 100 cc.	Urea ratio.	Phthalein elimination in 2 hrs.	Remarks.
1918	*cc.*	*gm.*		*gm.*	*mg.*		*per cent*	
Dog 17–227, weight 40 lbs.								
Apr. 22			9–10	0.144	30	4.8		
		20	10.15–11.15	1.726	121	14.2	71	Marked diuresis for several hours.
			11.15–12.15	1.526	104	14.6		
			12.15– 1.15	0.917	86	10.5		
" 24			10–11	0.100	87	1.16		9.15 a.m. Proteose injected.
	114	20	11.20–12.20	0.333	230	1.45	Mere trace in 3 hrs.	Intense intoxication. Marked oliguria in spite of much fluid intake and diuretics given.
			1.20– 2.20	Anuria.	223	0		2.25 p.m. Died in convulsions.
Dog 18–3, weight 36 lbs.								
Apr. 14			8.40– 9.40	0.132	28	4.7		
		20	10–11	1.981	129	15.3		
			11–12	1.491	121	12.3	75	Marked diuresis.
			12–1	1.024	96	11.3		
" 22			8.30– 9.30	0.120	26	4.6		9.45 a.m. Proteose injected. 11.50 a.m. Urea and dye injected. Moderate grade of intoxication.
	83	20	11.50–12.50	0.863	166	5.2		Mild diuresis.
			12.50– 1.50	0.695	161	4.3	42	
			1.50– 2.50	0.537	136	3.9		

TABLE IV.

Injection of Proteose. Effect upon the Ratio $\frac{\text{Urea in 1 Hr.'s Urine}}{\text{Urea in 100 Cc. of Blood}}$ *and the Elimination of Phenolsulfonephthalein. Urea Injected.*

	Proteose injected.	Urea injected.	Hour.	Urea excreted.	Blood urea per 100 cc.	Urea ratio.	Phthalein elimination in 2 hrs.	Remarks.
1918	*cc.*	*gm.*		*gm.*	*mg.*		*per cent*	
Dog 18–59, weight 48 lbs.								
Aug. 18		18	9–10	1.887	135	13.9		
			10–11	1.540	112	13.7	72	Marked diuresis.
			11–12	0.937	84	11.1		
" 24	90	18	1–2	0.997	195	5.1		10.40 a.m. Proteose injected.
			2–3	0.872	188	4.6	34	Moderate grade of intoxication. Mild diuresis. 1.37 p.m. 200 cc. of 10% dextrose.
			3–4	0.719	154	4.6		
Dog 18–85, weight 19 lbs.								
Mar. 15			9–10	0.119	24	4.9		
		12	10.15–11.15	1.596	119	13.5	80	Marked diuresis.
			11.15–12.15	1.264	108	11.7		
" 20			8.45– 9.45	0.121	27	4.5		10 a.m. Proteose injected.
	35	12	12–1	0.893	135	6.6	47	Mild grade of intoxication. Moderate diuresis.
			1–2	0.788	121	6.5		

Dog 18–59 (*Table V*).—Died during the night.

Autopsy.—All the organs intensely congested with dark purplish blood. Lumen of intestine contains considerable blood-stained watery mucus. Spleen very greatly enlarged and hemorrhagic. Kidneys show considerable postmortem softening. Pancreas partially digested.

Microscopic Examination.—Congestion of the viscera. Finer structures blurred by postmortem alterations.

TABLE V.

Dog 18–59. Large Female Pointer. Injection of Proteose. Effect upon the Ratio $\frac{\text{Urea in 1 Hr.'s Urine}}{\text{Urea in 100 Cc. of Blood}}$ *and the Excretion of Sodium Chloride.*

Sodium Chloride Injected.

Hour.	Chloride excreted.	Plasma chlorides per 100 cc.	Urea excreted.	Blood urea per 100 cc.	Urea ratio.	Remarks.
	gm.	*mg.*	*gm.*	*mg.*		
9–10	0.948	678	0.127	23	5.5	Moderate diuresis after salt injection.
10–11	0.600	663	0.142	24	5.9	
11–12	0.476	630	0.118	22	5.3	
Total.....	2.024		0.387			
Oct. 2, 1918, 10 a.m. 120 cc. of proteose solution intravenously.						
11.50–12.50	0.166		0.106	38	2.8	
12.50– 1.50	0.110	672	0.068	53	1.3	Intoxication intense. 12.30 p.m. 150 cc. of 10% sugar solution.
1.50– 2.50	0.090		0.032	64	0.5	Oliguria continued.
5–6	—*	631	0.020	87	0.2	Dye elimination from 6–8.30 p.m. mere trace. Dog died late during the night.
Total.....	0.366		0.226			

* Chlorides lost.

An experiment is tabulated in Table V showing the effect of proteose intoxication upon the excretion of injected sodium chloride. After a preliminary fast of 3 days, Dog 18–59, a female adult, weighing 45 pounds, was given 5 gm. of sodium chloride dissolved in 150 cc. of distilled water intravenously. The hourly excretion of chlorides was then determined for 3 successive hours. The plasma chlorides and the urea ratio were also determined. The next day the animal received 120 cc. of a dilute toxic proteose solution intravenously and 1 hour and 50 minutes later 5 gm. of sodium chloride in 250 cc. of distilled water also intravenously, following which the chloride excretion, the plasma chlorides, and the urea ratio were determined.

Reference to the table shows that the total excretion of chlorides following the injection of the proteose was markedly reduced. The plasma chlorides show only an insignificant rise in the 2nd hour above the level in the corresponding hour of the previous day. The plasma chlorides were not determined for each hour as in the control period because the large amounts of fluid injected with the view of helping the animal to survive the intoxication doubtless diluted the plasma to such an extent that figures on the chloride content would be of little value as far as their throwing light upon the question of salt retention is concerned.

The urea ratio was determined as a check on the grade of intoxication and the degree of kidney functional impairment as measured by the change in this ratio. It will be seen that this was lowered to between one-third and one-fourth of its normal value, which fact speaks for a very intense intoxication. However, the decrease in total chloride excretion over the period is much greater than is the decrease in the urea ratio.

Dog 19–29 (Table VI).—

Autopsy.—Heart and lungs normal. Liver and spleen extremely engorged. Subcapsular hemorrhages in spleen. Gall bladder markedly congested; wall appears like jelly—very edematous and thick. Pancreas very hyperemic. Kidneys slightly engorged, but otherwise negative. Intestinal tract less engorged than usual.

Microscopic Examination.—Kidneys normal. Moderate congestion of other organs.

In Table VI are presented two experiments similar to that tabulated in Table V.

Dog 19–29, a small female adult fox-terrier, weighing 13 pounds, after a preliminary fast of 3 days, received 5 gm. of sodium chloride in 200 cc. of distilled water intravenously Oct. 12. The chloride excretion was then determined for the next 24 hour period subdivided into the following periods: first 2 hours; second 2 hours; next 4 hours; and finally the remainder of the 24 hours. The blood urea was determined for the first two subperiods merely to find the normal level for comparison with the values to be obtained in the later experiment. The plasma chlorides were determined at the end of the 1st hour after salt injection and again the next morning near the end of the 24 hour period.

The animal was fed a mixed diet on Oct. 13 and 14, after which the food was withdrawn for a second preliminary fast of 3 days. On Oct. 18, at 10.10 a.m. 26 cc. of a toxic proteose preparation were injected intravenously and at 11.10 a.m., 5 gm. of sodium chloride in 250 cc. of distilled water were given intra-

venously. The chloride excretion, plasma chlorides, and the blood urea were then followed over a 24 hour period subdivided as in the above control period.

The intoxication was very intense, the animal dying about 28 hours after the injection of the poison. The total excretion of chlorides was much reduced. The blood urea mounted to 93 mg. per 100 cc. of blood, even after some dilution by injected fluid. The plasma chloride is slightly higher at the end of the 1st hour after the salt injection and considerably higher near the end of the period than at corresponding times during the preliminary control period.

TABLE VI.

Injection of Proteose. Chloride Excretion. Sodium Chloride Injected.

	Hour.	Chloride excreted.	Plasma chlorides per 100 cc.	Blood urea per 100 cc.	Remarks.
1918		*gm.*	*mg.*	*mg.*	
Dog 19–29, weight 13 lbs.					
Oct. 12	9.15–11.15	1.806	684	24	Moderate diuresis.
	11.15– 1.15	1.029	644	23	
	1.15– 5.15	0.670			
	5.15 p.m.–9.15 a.m.	1.848	611		Next day.
	Total 24 hrs.....	5.353			
	Oct. 18, 10.10 a.m.	Proteose injected intravenously.			
" 18	11.10– 1.10	0.341	689	36	Moderate grade of intoxication at first.
	1.10– 3.10	0.227		53	Slight diuresis during 1st few hrs. Oliguria during latter portion of period.
	3.10– 7.10	0.208		73	Intoxication intense later. 200 cc. of 6% acacia and 10% glucose intravenously.
	7.10 p.m.–11.10 a.m.	0.258	661	93	Next day. Rectal temperature subnormal (34°C.) a few hrs. before death. Oct. 19, 1.45 p.m. Killed in terminal stage of intoxication.
	Total 24 hrs.....	1.034			

TABLE VI—*Concluded.*

	Hour.	Chloride excreted.	Plasma chlorides per 100 cc.	Blood urea per 100 cc.	Remarks.
1918		*gm.*	*mg.*	*mg.*	
Dog 19–42, weight 28 lbs.					
Oct. 6	10.13–11.13	0.822	670		
	11.13– 1.13	0.842	661		Considerable diuresis.
	1.13 p.m.–10.13 a.m.	3.328	561		Next day.
	Total 24 hrs.....	4.990			
	Oct. 11, 10 a.m. 27 cc. of proteose injected intravenously. 12 m. 5 gm. of NaCl + 10 gm. of urea in 250 cc. of distilled water.				
" 11	12–1	0.743	670		Mild grade of intoxication. Reaction somewhat delayed.
	1–3	0.606	663		Mild diuresis.
	3 p.m.–12 m.	1.750	611		Next day.
	Total 24 hrs.....	3.099			

Dog 19–42 (Table VI) was given intravenously 5 gm. of sodium chloride dissolved in 250 cc. of distilled water containing 10 gm. of urea at 10.13 a.m., Oct. 6, after a preliminary fast of 3 days. The chloride excretion and the plasma chlorides were then determined for the next 24 hours divided into three subperiods; *viz.*, for the 1st hour after the salt injection; for the next 2 hours; and finally, for the remainder of the 24 hour period. Blood samples for the determination of the plasma chlorides were taken in the middle of each of the first two periods and near the end of the third.

The animal was then fed a mixed diet until Oct. 8, after which the food was withdrawn.

At 10 a.m., Oct. 11, 27 cc. of toxic proteose solution were injected intravenously.

At 12 m., 5 gm. of sodium chloride plus 10 gm. of urea dissolved in 250 cc. of distilled water were given intravenously exactly as in the control period. The chloride excretion and the plasma chlorides were then determined over a 24 hour period subdivided as for the control period.

The urea was injected merely as an added diuretic to insure a sufficient flow of urine.

Even in this mild intoxication there is seen to be a moderate retention of chlorides, more striking for the third subperiod than for the first two. The plasma chlorides for the second subperiod are slightly higher and the next morning considerably higher than at the corresponding times during the control period. Yet, the difference is small and may not be of great significance in comparison with the marked decrease in chloride excretion.

Table VII groups together the experiments on the effect of proteoses from miscellaneous sources upon the blood urea and the elimination of phenolsulfonephthalein. It will be seen from the figures presented that neither the blood urea nor the percentage of dye excreted is much disturbed by comparatively large doses of most of the products used. The various digests were tested at different stages, but, since the results were uniform from time to time, a few representative figures only are tabulated here. These are a unit as far as they go in showing no depression of the renal function.

Most of the products examined possess but slight toxicity as compared with the proteose preparation from the obstruction material. One only produced symptoms of intoxication, when injected intravenously in the dog, comparable with the latter material. This was obtained from a pepsin digest of muscle tissue which contained a rather luxuriant growth of bacteria, including some proteolytic anaerobes, which were able to grow under the thick layer of toluene intended to prevent bacterial growth. Besides a rich content of alcohol-soluble substances which resist heat at boiling temperature and which produce an anaphylactic-like reaction, this particular digestion mixture also contained an alcohol-insoluble substance or group of substances which gave rise to symptoms closely resembling those produced by an injection of the toxic proteose from the contents of an obstructed small intestine or a closed intestinal loop.

The small amount of this alcohol-precipitable material injected for the renal function study caused but a slight decrease in the percentage of dye eliminated, which itself may have been due in large part to extrarenal factors since none of the precautions taken in the majority of the experiments above (Tables I and II) against these was exercised in the case of the experiments presented in Table VII.

Too little is known as yet regarding the nature and the production of this toxic preparation to discuss it further here. It is being inves-

TABLE VII.

Injection of Proteose Preparations from Various Sources.

Source of proteose.	Dog No. and weight.	Quantity of solution injected.	Clinical reaction.			Blood urea per 100 cc.	Phthalein elimination in 2 hrs.	Remarks.
			Pulse.	Body temperature.	Miscellaneous.			
		cc.		°C.		*mg.*	*per cent*	
Commercial Witte's "Peptone."	19–59 49 lbs.	75	Greatly depressed.	38.2 39.2	Dog slightly more dull..	38	64	3.2 gm. dry weight. Dog soon recovered.
	19–50 27 lbs.	90	Greatly depressed.	38.2 38.7	Dog slightly more dull.	44	67	4 gm. dry weight. Dog soon recovered.
Pepsin digest of liver tissue (Martin's broth).	19–42 26 lbs.	200	Slight transient depression.	38.1	Bright and active.	29	71	Considerable proteose content.
Pepsin digest of sterile muscle tissue.	19–51 28 lbs.	55	Slight transient depression.	38.6	Bright and active.	33	65	Rich in proteoses.
Pepsin digest found to contain proteolytic bacteria.	19–30 22 lbs.	30	Moderate depression lasting several hrs.	38.0 40.1	Very dull. Vomiting. Diarrhea.	38	58	Proteose precipitated with alcohol.
Sterile heart muscle digested by *B. histolyticus*.	19–51 27 lbs.	40–70	Moderate transient depression.	38.0 39.4	Slightly dull.	30	68	Toxicity slight. Proteose content high.
Sterile heart muscle digested by *B. sporogenes*.	19–50 26 lbs.	40–70	Moderate transient depression.	38.0 39.8	Slightly dull.	32	69	Toxicity slight. Proteose content high.
Sterile heart muscle digested by *B. bifermentans*.	19–51 27 lbs.	30–62	Moderate transient depression.	38.4 39.4	Quiet. Salivated.	34	72	Toxicity slight. Proteose content high.

tigated from various angles at present, including a further study of its chemical properties and the bacterial flora concerned in its production.

Table VIII shows the effect of a comparatively large dose of Witte's peptone solution on the urea ratio and the elimination of phenolsulfonephthalein. In order that the effect of the proteoses from this source might be compared with that of the toxic proteose obtained from the obstruction material, the determinations were made 2 hours after the

TABLE VIII.

Dog 19–59. Injection of Witte's Peptone. Effect upon the Ratio $\frac{\text{Urea in 1 Hr.'s Urine}}{\text{Urea in 100 Cc. of Blood}}$ *and the Elimination of Phenolsulfonephthalein.*

Date.	Hour.	Urea excreted.	Blood urea per 100 cc.	Urea ratio.	Phthalein elimination in 2 hrs.	Remarks.
1918		*gm.*	*mg.*		*per cent*	
July 9	10–11	0.167	33	5.0		No food on previous day.
	11–12	0.172	34	5.0	72	
July 10	10–11	0.124	24	5.1		No food for 2 days.
	11.30 a.m. 4 gm. of Witte's peptone dissolved in 80 cc. of water given intravenously.					
	1.30–2.30	0.240	38	6.3		Blood pressure only slightly below normal. Animal bright and active.
	2.30–3.30	0.232	36	6.4	70	

peptone solution was injected, when the animal, therefore, had almost completely recovered from the effects of the injection. There is seen to be a slight increase in the blood urea and in the quantity of urea excreted per hour. In contrast to the observations made on the relation of the urea excreted per hour to the blood urea in the typical proteose intoxication, there is a slight rise in the ratio, which indicates a normal response on the part of the kidneys. The phthalein elimination is likewise normal.

DISCUSSION.

The outstanding fact presented in the foregoing tables is that the excretory function of the kidneys is decidedly impaired by the intravenous injection of the toxic material obtained from the contents of the obstructed small intestine.

Another interesting feature of these experiments is that the effect of the toxic substance on the excretory mechanism was found to be only temporary, in most instances disappearing soon after the animal had recovered completely from the intoxication. The suggestion made in the previous report that the degree of impairment of the renal function corresponds fairly closely with the grade of intoxication is further substantiated by these observations.

The demonstration of the latter points explains the observation (8) that the apex of the curve of nitrogen excretion occurs usually on the 2nd day following an injection of toxic proteose instead of on the 1st day when the blood non-protein nitrogen is at its highest level.

The experiments tabulated here likewise explain the enormous increase in the non-protein nitrogen of the blood sometimes observed after proteose injections as being due in part to the retention of this group of substances as well as to an increase in the rate of protein breakdown.

That delay in the absorption of the test dye during the period of intoxication plays no prominent part in the observed failure of the kidneys to eliminate this substance at the normal rate is clearly shown by the experiments presented in Table II in which the phthalein was injected directly into the blood stream. Since comparatively large amounts of fluid were administered during the period of observation following the proteose injections, it seems to us improbable that the factors of lowered blood pressure and concentration of the blood commonly observed in this condition can account for any large part of the temporary defect in the eliminative function.

Most of the other proteose preparations employed in this study fail to cause any appreciable impairment of the renal function as far as they were examined, although they give the same chemical tests given by the proteose material procured from the obstructed intestine. The initial depression of the blood pressure produced by

these miscellaneous proteoses was in every case greater than that caused by injection of the obstruction material. Therefore, this factor can also be dismissed as having no important place in the explanation of the observed facts.

It is evident that all proteoses do not depress the renal function, although some which were tested by Chittenden, Mendel, and Henderson (12) were found to cause anuria without materially affecting the blood pressure and may well have temporarily disturbed the renal function.

We believe that it is more than a coincidence that this proteose mixture obtainable from the obstructed small intestine, but not from the normal intestine, can effect so pronounced a depression of the renal function. Furthermore, the acute intoxication of intestinal obstruction is accompanied by a similar depression of renal function which is likewise transient and capable of swift return to normal if the intoxication is relieved. This is strong evidence that substances resembling exactly those found in the obstructed intestine are wholly responsible for the general intoxication of intestinal obstruction.

There appears to be produced by these toxic substances a primary injury of the secreting cells of the kidney itself which is responsible for the greater part of the impairment as was pointed out in the preceding communication (1), although this injury is not demonstrable by the ordinary histological technique. As far as we are aware this is one of the first instances observed in which a marked kidney injury or impaired function has been demonstrated, followed very quickly by repair and a return to normal, associated with no trace of permanent injury. It seems probable that this type of injury might prove to be more serious if superposed upon a kidney already diseased (chronic nephritis).

SUMMARY AND CONCLUSIONS.

The injection of the toxic proteose obtained from the contents of the obstructed small intestine causes a definite impairment of the eliminative function of the kidneys as shown by a decreased capacity to excrete urea, sodium chloride, and phenolsulfonephthalein.

This involvement of the renal function is similar to that shown by the preceding report (1) to accompany the intoxication of intestinal obstruction.

The observed depression of function is readily demonstrable even when large amounts of fluid and urea, dye, or salt are injected directly into the blood stream.

There is in all probability a temporary injury of the kidney cells, since the most important extrarenal factors have been largely eliminated in the above experiments.

There is no appreciable impairment of the renal function following the injection of a number of other proteose preparations from a variety of sources.

This study affords new evidence in favor of the view that the function of an organ can be profoundly disturbed for a time without any demonstrable anatomical lesions.

The repair of this type of injury promptly follows the disappearance of the intoxication and is functionally and anatomically perfect.

In conclusion the writers take pleasure in the acknowledgment of aid and cooperation on the part of Dr. H. M. Evans of the Department of Anatomy. Work on vital staining of the kidney and body tissues in conditions of proteose intoxication was taken up by Mr. McQuarrie, working under the supervision of Dr. Evans.

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